CHARACTERISING FACTORS PREDICTIVE OF INFECTION IN SEVERELY INJURED PATIENTS

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STATEMENT OF ORIGINALITY

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ABSTRACT

Infection after trauma complicates the patients clinical course. Infection leads to longer critical care and hospital stays, has been associated with increased mortality rates and places considerable cost pressures on health economies. The predictors of infection after severe injury are not known, and the effects on outcomes other than mortality are under-reported.

The overall objective of this research was to characterise factors predictive of infection in severely injured patients admitted to critical care. A prospective cohort study of 271 patients investigated admission factors predictive of the development of infection. A second study of 280 patients evaluated post-injury immune cell changes and the association with infection. Thirdly the relationship between early coagulopathy and infections was investigated in 158 patients. Finally a study of 385 patients examined the use of Tranexamic Acid (TXA) and its association with infection and other outcomes.

Infection was a significant burden for severely injured patients. Admission hypoperfusion was the only early characteristic associated with the development of infection, and a dose dependent relationship was observed between severity of shock and increased percentage of infection ($p<0.01$). Lymphopenia prolonged to day four post injury was strongly predictive infection (OR 0.10, CI 0.02-0.48, $p<0.01$). At 24 hours, the anticoagulant Protein C was lower in those with infection (Infection: 70.2 iu/dL vs. No infection: 83.3 iu/dL $p=0.02$), and increased fibrinolysis was also associated with infectious complications (Infection: 6156 µg/L vs. No infection: 3324 µg/L $p=0.03$). There was a trend to a beneficial relationship between TXA and infection, and it was independently associated with reduced organ failure (OR 0.27, CI: 0.10 – 0.73, $p=0.01$) and mortality (OR 0.16 CI 0.03 - 0.86, $p=0.03$).

In severely injured patients, admission shock, prolonged lymphopenia and early coagulation dysfunction post severe injury were independent predictors of infection. Timely modulation of these responses after trauma may help to reduce the burden of infection.

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Publications

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Cole E, Davenport R, Willett K, Brohi K. The burden of infection in severely injured trauma patients and the relationship with admission shock severity. J Trauma Acute Care Surg. 2014 Mar; 76(3):730-5.

Cole E, Davenport R, De'Ath H, Manson J, Brockamp T, Brohi K. Coagulation system changes associated with susceptibility to infection in trauma patients. J Trauma Acute Care Surg. 2013 Jan; 74(1):51-7; discussion 57-8.

Presentations

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ABBREVIATIONS

AIS	Abbreviated Injury Score
ANOVA	Analysis of Variance
APTT	Activated Partial Thromboplastin Time
AT	Antithrombin
ATC	Acute Traumatic Coagulopathy
BD	Base Deficit
CA	Clot Amplitude
CCU	Critical care unit
CDC	Center for Disease Control
CI	Confidence Intervals
CT	Clotting time
DD	D-Dimer
ED	Emergency Department
ELISA	Enzyme-linked Immunosorbant Assay
FFP	Fresh Frozen Plasma
GCS	Glasgow Coma Scale
ICU	Intensive Care Unit
INR	International Normalised Ratio
IQR	Interquartile Range
ISS	Injury Severity Score
LOS	Length of Stay
MCF	Maximum Clot Firmness
mEq/L	Milliequivalents per Litre
MHP	Major Haemorrhage Protocol
Mins	Minutes
ml	Millilitre

mmol/L	Millimoles per Litre
MODS	Multiple Organ Dysfunction Syndrome
MOF	Multiple Organ Failure
ng/mL	Nanogram per millilitre
OR	Odds Ratio
pg/mL	Picogram per millilitre
pLAR	Professional Legally Appointed Representative
PAI-1	Plasminogen Activator Inhibitor-1
PAP	Plasmin α2-anti Plasmin
PC	Protein C
PICS	Persistent Immunosuppression Catabolism Syndrome
PRBC	Packed Red Blood Cells
PS	Protein S
PT	Prothrombin Time
ROTEM	Rotational Thromboelastometry
RR	Relative Risk
SBP	Systolic Blood Pressure
SD	Standard Deviation
SIRS	Systemic Inflammatory Response Syndrome
SOF	Single Organ Failure
SOFA	Sequential Organ Failure Assessment (score)
TXA	Tranexamic Acid
µl	Microlitre
vs.	Versus
VFD	Ventilator Free Days
VTE	Venous Thromboembolism
WBC	White Blood Cells
WHO	World Health Organisation

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CHAPTER ONE INTRODUCTION

1.1 The disease of trauma

1.1.1 The burden of traumatic injury

Traumatic injury is a major contributor to the global burden of disease (1). In many countries, trauma is the leading cause of mortality in individuals aged up to 45 years (2), and in England and Wales 14,000 people die annually as a result of injury (3). Trauma is also the cause of significant morbidity, and many patients who survive their injuries may suffer in-hospital adverse events such as infection and organ failure (4, 5). Despite improvements in injury prevention and clinical management, trauma is still responsible for over 80 million lost disability-adjusted life years (DALYs). This is higher than ischaemic heart disease, diarrhoeal disorders and HIV infection (6). Longer term physical, psychological and social impairments following trauma are well recognised (7), and injury is the leading cause of disability in adults of working age (8, 9).

The management of trauma also represents a serious financial burden to the health economy. In Britain, it is estimated that the average in-hospital cost of a severely injured patient is £9,530 (10, 11). However, this figure does not include the costs of rehabilitation and continuing care following discharge. The National Audit Office (NAO) estimates health expenditure for complex trauma management to be around £0.3-0.4 billion annually, and suggests that the burden of severe injury on economic output could be up to £3.7 billion (12).

1.1.2 Epidemiology of traumatic injury

Traumatic injury causes 10% of deaths globally and is the leading cause of mortality from the ages of 5 to 44 years in developed countries (13, 14). The majority of trauma related deaths occur early in the patient's course, either in the pre-hospital phase or in

the acute in-hospital period (15-17). Injury to the central nervous system and uncontrollable haemorrhage are the leading causes of early death (18-21). Subsequent in-hospital morbidity and mortality is predominantly attributed to the development of multiple organ failure (22, 23) and overwhelming infection or sepsis (5, 24). The risk of death in trauma patients remain higher than matched non-injured individuals for up to ten years post injury (25).

Traumatic injury can be caused by blunt or penetrating force, or a combination of the two. Blunt injuries include falls, road traffic collisions and crush injuries whilst penetrating trauma involves gunshot injuries, stabbing or impalement. Blasts result in both blunt and penetrating injuries (26, 27), as may intentional trauma due to assault or deliberate self-harm (28).

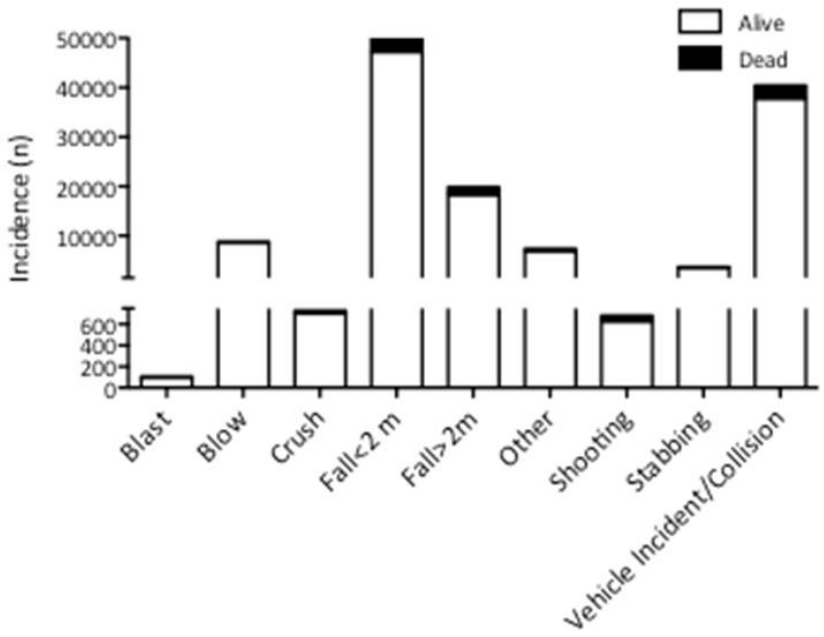


Figure 1.1 A summary of trauma in England and Wales from 2002 to 2011. 'Blow' includes blunt assaults, 'other' relates to injuries sustained following industrial and agricultural incidents. (Figure courtesy of the Trauma Audit & Research Network).

Internationally, the predominant cause of injury in civilian populations is blunt trauma (16-18). This is true for the United Kingdom (UK), where the most common mechanism

of injury is falls (3) (Figure 1.1).

1.1.3 Measuring injury severity

The severity of injury is defined using the injury severity score (ISS) (29). This score is calculated retrospectively once all injuries have been diagnosed. ISS is an anatomical scoring system which divides the body into six regions, each assigned an abbreviated injury score (AIS) from 0 to 6. The three highest AIS scores are squared and added together to produce the ISS. An ISS ≤5 indicates minimal injury; an ISS ≥15 suggests severe injury whilst an ISS ≥25 signifies critical injury (29). On the whole, increased ISS is associated with poorer outcome, both in terms of mortality and morbidity (30, 31). However, the physiological response after trauma is complex and may also influence outcome (32). Therefore the main limitation of the ISS is that it is an anatomical scoring system which does not take physiological derangement into consideration. Nevertheless, the ISS provides an internationally recognised score used to consistently convey severity of injury.

1.1.4 Trauma systems and processes of care

There is a large body of evidence which has demonstrated that organisation of regional trauma systems is strongly associated with better patient outcomes (33-35). Trauma systems are also of benefit to society by virtue of the gain in costs per life saved (36). In the UK, numerous reports published over the past 25 years have identified widespread deficiencies in the provision of trauma care (37-40). Following the publication of the *Healthcare for London: A Framework for Action* (41) in 2007, the UK National Confidential Enquiry into Patient Outcome and Death (NCEPOD) published '*Trauma: Who Cares*?'. This revealed inadequacies in the quality and processes of care for 60% of severely injured patients. (42). This population-wide analysis of trauma care highlighted an imperative for organised trauma networks in the UK. Accordingly, in April 2010 Greater London introduced a pan-regional inclusive trauma system.

An inclusive system is designed to care for all injured patients in a specified geographical location, with collaboration between pre hospital care, hospital services, community facilities and local government (43). Resources are organised to deliver optimal care at the most appropriate facility at the right time (44, 45). Using a hub and spoke model, institutions designated as Major Trauma Centres (MTCs) are specialty hospitals with a full complement of clinical disciplines available on site, responsible for treating the most severely injured patients (46). Smaller trauma units, supported by the MTC, will care for less injured patients within the system.

Leadership provided by senior clinicians experienced in trauma management is associated with improvements in timing and quality of care processes (47). Standards for processes such as time to diagnostic imaging or major haemorrhage management aim to guide early trauma resuscitation. However, the quality of evidence for processes of care is variable (7, 48), and the association with outcomes other than mortality or length of stay is poorly described (49, 50). An important challenge for contemporary trauma care is to identify which early clinical processes translate into improved patient outcomes. This will be best achieved through robust research studies.

1.1.5 The need for trauma research

Despite the global impact of traumatic injury, published evidence to guide management is limited, primarily due to a lack of research in this field. With the exception of a small number of established research centres in Europe and the USA, the majority of civilian injury research comprises data reviews from trauma registries (51).

Throughout history, wars and conflict have resulted in advancements in trauma management (52). In 2005, a military ethical human research protection plan (HRPP) was drafted, facilitating research in a combat environment (53). This has enabled multisite, prospective research studies in combat zones such as Afghanistan and Iraq (54). Whilst there are differences in populations and injury mechanisms, current military

studies have influenced trauma research and management in civilian settings (55), in areas such as haemorrhage management and transfusion practices (56-59).

There are however, a number of challenges for conducting civilian trauma research, and efforts have been hindered by the complex nature of trauma care and heterogeneous populations (2). There may be difficulties in enrolling patients who present unpredictably, in physiological extremis, and outside of traditional working hours. Obtaining consent in patients who may be unconscious, impaired or distressed due to injury adds to the challenge (60). Additionally, trauma patients may be mobile, injured away from home or transient within an area (51), and following up those who may not live close to the trauma system may be difficult after discharge.

Underpinning these challenges are low levels of investment into trauma research (61). The RAND corporation report concluded that less than one percent of the total UK public expenditure on health research is allocated to trauma (62). Nevertheless, despite these acknowledged challenges, recent clinical trials and good quality observational studies have been conducted successfully (63-65). Trauma research is essential, in order to develop and assess new treatments, improve clinical care, and strengthen trauma systems (2).

1.1.6 Measuring outcomes after trauma

Outcome measurement following traumatic injury is important to many key stakeholders, including clinicians, healthcare systems, researchers and the public. The heterogeneous nature of traumatic injuries and the numerous clinical interventions required by this patient population means that outcome measurement is complex (6). Outcomes are measured in trauma research to evaluate the effect of an intervention or process. The challenge for current research is to identify the optimal, most sensitive outcome measure (or surrogate of outcome) for use in trials and clinical studies. The

primary outcome measure in the majority of published trauma research is mortality. Whilst this is unambiguous and consequently feasible to quantify, it can be affected by many variables such as age, injury severity or pre-existing morbidity. Therefore, mortality may not be a very sensitive measure of process, intervention or the patient experience following injury.

System based outcomes such as critical care stay, ventilator use, or length of hospital stay are also commonly reported. Again, there may be a number of variables, such as differing admission or discharge criteria, which could influence these outcomes. Other in-hospital or intermediate measures of outcome include development of adverse events such as infection, organ failure or cardiac dysfunction (66-69). These too are subject to variability in trauma systems, although the publication of internationally validated measures such as organ injury scores or infection criteria means that the evaluation of in-hospital outcomes is becoming increasingly more reliable. Given that mortality and length of stay are affected by many non-adaptable variables, morbidity outcomes may provide more sensitive measures of clinical intervention and the in-hospital patient experience (6) and as such are useful tools for evaluation of trauma care.

1.2 Infection and Trauma

1.2.1 The significance of infection in trauma patients

Infectious complications have previously been recognised as an adverse clinical outcome following trauma (70-72). Infection leads to increased critical care admissions, longer lengths of critical care and hospital stay, and has been associated with increased mortality (5, 72, 73). Patients who survive infection to hospital discharge are reported to have decreased functional status and increased healthcare use up to a year after injury (74). Infections also place considerable cost pressures on health care providers and hospital acquired infections continue to raise safety concerns for patients (75-77) .

There is a lack of evidence however, on the determinants of infectious complications in contemporary trauma care. Studies examining the specific early characteristics that predispose injured patients to infection are limited. The majority of literature has focused on specific micro-organisms or location of infection (78-80), rather than the burden of trauma related infection as a whole.

1.2.2 The incidence of trauma infection: the clinical evidence

There are few clinical studies investigating the relationship between trauma and the overall burden of infection. Where studies exist, more than half are derived from retrospective registry reviews. Retrospective database and registry reviews can yield large numbers of patient data for research; however this method may fail to account for unmeasured variables that were not collected during initial data collection.

The reported incidence of trauma related infections is variable. Historical evidence from large registry reviews reported a relatively low incidence of infection at 16 and 17% respectively (81, 82). These studies associated infection in trauma patients with invasive therapeutic interventions. Subsequent retrospective evidence reported a lower incidence of 9%, with trauma infections predominantly related to older age, injury severity and operative intervention (70). Almost a decade later, a large prospective study examined the risk factors associated with infection in patients admitted to a Canadian trauma system (71). At 37%, the incidence of infectious complications was much higher than the previously published evidence. In this cohort study, infections most commonly affected the respiratory and urinary tracts (28% and 24% respectively). Multivariate regression analysis demonstrated independent relationships between infection and spinal injury (Odds ratio: 5.0, CI: 1.8-15.0; $p<0.001$), ventilation (Odds ratio: 2.6, CI: 1.5-4.4; $p<0.01$), multiple surgical interventions (Odds ratio: 2.8, CI: 1.1-6.7; $p=0.02$), and multiple blood transfusions (Odds ratio: 2.9, CI: 1.8-5.0; $p<0.01$). However, patients who received an early (<24hours) operative intervention had a significantly lower risk of infection (Odds ratio: 0.37, CI: 0.20-0.68; $p<0.001$). Contemporary early trauma management has changed since this research was

conducted. Timely haemorrhage control with early operative or radiological intervention is now a central tenet of injury care. The reduced infection rates associated with early operations in this Canadian study may have reflected a protective relationship between timely haemorrhage management and decreased inflammatory responses.

More recently, retrospective reviews suggested that the incidence of post-traumatic infection was between 6-10% (66, 83-85). Whilst this represents a relatively low burden of trauma-related morbidity, it may illustrate the difficulty in reporting infection data on patients with differing degrees of injury severity. In patients whose injuries were severe enough to require intensive care admission (86), rates of infection were much higher at 45%. Prolonged ventilation (Odds ratio: 12.8, $p<0.001$) and invasive devices (Odds ratio: 4.6, $p<0.001$) were independently associated with the development of infection. However, the majority of individuals (72%) had single traumatic brain injuries, and no analysis of the patients' injury severity, haemodynamic status or resuscitation intervention was provided. It is difficult therefore to determine if the high incidence of infection reported for this cohort is representative of all multiply injured trauma patients.

Penetrating injury is a significant cause of trauma-related morbidity, and the resulting open wounds may increase the incidence of infectious complications. Evidence suggests that both penetrating and blast injuries seen in civilian and military trauma have resulted in infection rates of up to 50% (84, 87, 88). Penetrating hollow viscous injury in particular, is strongly associated with the development of surgical site infections (89), whilst infection rates in open wounds and fractures are as high as 50% in some orthopaedic series (90). The significant incidence of infection following penetrating injury has resulted in number of prophylactic management guidelines for traumatic wounds and open fractures (91, 92). Conclusive high quality evidence of the efficacy of these recommendations is yet to be published (92-94).

Pneumonia continues to be the most common infection reported in trauma studies. The development of pneumonia in trauma patients has been associated with increased age (66, 83), pre-existing co-morbidities (84) and spinal cord injury (85). The burden of ventilator associated pneumonia (VAP) has resulted in a number of prospectively conducted studies. Prolonged ventilation (95), early clotting dysfunction (96), blood transfusion (97) and severe thoracic injury (98) have all been independently associated with VAP. The need for strict infection prevention and control has also been reported (99-101), and subsequently, critical care bundles have been introduced to minimise the risk of VAP (102). Recent evaluation suggests that these interventions have led to a decreased incidence of this specific type of infection in trauma patients (103-105).

Prospective research into the risks of VAP has provided translational evidence for clinical practice which has led to better outcomes for patients. However there is a knowledge gap in relation to the predictors of all infections in the most severely injured patients. Whilst some studies exist, risks have yet to be comprehensively described in prospective evidence. Characterisation of clinically identifiable risks may enable the development of prediction tools to guide the management of severely injured trauma patients.

1.2.3 Risk factors associated with infection following trauma

Some of the most prevalent evidence reporting risks of infection following injury has emanated from a group in Maryland USA. In common with earlier studies, older age was found to increase the burden of trauma infection (106). Patients over 65 years old had a two-fold greater infection rate than those <65 years, and this correlated with increased mortality and length of stay. In this cohort of patients, only COPD was found to be independently related to infection.

In the same year, admission systemic inflammatory response syndrome (SIRS) was found to be an independent predictor of infection following blunt trauma (107). After

adjusting for age and injury severity, hypothermia and leukocytosis on admission were strongly associated with infection, increased length of stay and mortality. A subsequent study by this group reported that persistent SIRS was also found to be highly predictive of infectious complications (108). Persistent SIRS was defined as a SIRS score of ≥2 daily from day three to seven post injury. SIRS during these time points was associated with a significantly increased risk of infection (SIRS present day 7 - Relative risk: 2.81, CI: 1.78-4.48; $p<0.001$). Further evaluation of this relationship was conducted, where the definition of persistent SIRS was extended to day 21 post injury. SIRS was still present in 50% of patients at three weeks post injury, and at this time point there was a 25-fold increased risk of infection (Odds ratio: 24.7, CI: 11.2-54.0; $p<0.001$) (109).

Whilst these SIRS studies add to the body of knowledge about trauma-related infections, there are a number of limitations. SIRS is common following traumatic injury, and a number of variables influence its presence. None of the studies discuss the haemodynamic status of the patient; therefore haemorrhage, shock, use of blood products or other potential causes of inflammation were not included in the analysis. The findings do not explain if SIRS was constant during the time from injury, or whether the persistent SIRS was in fact a second or third episode which may have been caused by operative intervention or an infectious episode. Furthermore, the temporal relationship between the development of SIRS and diagnosis of infection was omitted from these studies. A final limitation of this work is that analysis of immune cells other than leukocytes was not conducted, which may have revealed a more about the inflammatory response to trauma.

Evidence of the relationship between trauma haemorrhage and the development of infection is very limited. There are a small number of studies that report an association between trauma-related infection and administration of blood products. A dose dependent relationship was found between red cell transfusion and post trauma infection (110). Adjusting for age and injury severity, multivariate regression confirmed an independent association between packed red cells and the development of infection

(Odds ratio: 1.08, CI: 1.02-1.14; p<0.01). Red cell transfusions per patient ranged from 0 to 46 units, suggesting a degree of uncontrolled haemorrhage in some patients, however this factor was not included in the analysis. A similar study reported that the odds of infection were eightfold greater in transfused patients (Odds ratio: 7.9, CI: 2.3-27.5; p < 0.001) compared to matched controls having received no blood, independent of age and ISS (111). Patients who received blood were significantly more shocked and coagulopathic on arrival to hospital, yet neither of these potentially confounding variables were considered in the evaluation of results.

Conversely, two studies suggest that it is not the volume of red cell transfusion that contributes to an infection risk, but the age of blood products used after trauma. A small retrospective review of 61 severely injured patients (ISS>15) matched for age, injury severity and admission hypoperfusion reported an increased association between transfusion of blood greater than 14 days old and infection (Odds ratio: 1.12, CI: 1.01-1.26, p=0.03) (112). These findings were confirmed in a larger cohort of 196 severely injured patients, who suffered a 29% incidence of infection. After adjusting for ISS, operative interventions and antibiotic use, older blood was independently associated with infection (Odds ratio: 1.03, CI: 1.01-1.07; p=0.03) (113). Biochemical changes occurring during prolonged red cell storage (leading up to expiry) result in activation of the immune system (114), which may explain the increased infection risk in these studies.

Finally, elevated body mass index (specifically BMI> 30), has been linked to increased infectious complications following injury (115). Obese patients had a five-fold increase in infections and furthermore, morbid obesity resulted in a six-fold increased risk. Uncontrolled glycaemic control post injury in obese and diabetic patients was also strongly predictive of infection (67), with a dose dependent increase in infections associated with rising blood glucose. Whilst these findings are important in understanding potential risk of infection, obesity may be a complicating factor for only a

limited number of trauma patients. Other more common patient, injury or physiological variables may be more predictive of infection risk for the majority.

Infection prevention and control processes are an important part of contemporary trauma management, yet the risk of infection is still considerable. Invasive interventions and mechanical ventilation are established factors for increased risk of infection, and care bundles help to mitigate this. However the evidence has yet to identify predictors of all types of infection in a severely injured population. Inflammatory changes have been implicated in the development of infectious complications; though the published research is limited to evaluation of early and persistent SIRS responses. Analysis of other immune system changes following trauma may add to this knowledge. Evidence relating to haemorrhage and coagulopathy as drivers of trauma infections is predominantly restricted to the association with blood transfusions. The involvement of trauma haemorrhage and coagulopathy in the development of infection in severely injured civilian patients remains uninvestigated.

1.2.4 Infection after trauma: the military evidence

Combat related injuries are complicated by higher rates of infection. Blasts cause both blunt and penetrating forces resulting in wounds, soft tissue injury and mangled extremities, and are strongly associated with infection (87, 116-118). As a result of recent conflicts, military trauma studies have led to a renewed focus on post-injury infectious complications (119). Generally, sites of combat trauma infection and specific microbes differ from civilian reports (118, 120), and specific contemporary conflict mechanisms such as blasts caused by improvised explosive devices, tend to confer a high risk of infection, (87, 118).

Trauma care in a military setting has obvious differences to civilian trauma systems. Initial management may start in the field and move through increasing levels of care

until the patient is repatriated back to a home based hospital. The number of environmental, physiological and clinical challenges this presents for trauma infection control have resulted in the *Guidelines for the Prevention of Infections Associated With Combat-Related Injuries* (121). A current ongoing study aims to monitor adherence to these guidelines and evaluate outcomes in military trauma patients with infection. The Trauma Infectious Diseases Outcomes study (TIDOS) is a five year observational investigation retrospectively recruiting patients who developed infection during various echelons of military trauma care (119). Prospective follow up is conducted at pre-specified time points over five years following discharge. Initial analysis from the first year of study reported a 27% infection rate, with a predominance of wound infections, pneumonia and osteomyelitis. Findings suggested that patients who develop infection tended to be more extensively injured requiring prolonged hospital stays. More detailed outcomes analysis is yet to be reported. This study will encompass significant numbers of participants yielding contemporary data to add to the body of knowledge on trauma infections. The challenge will be to extrapolate combat zone practice and infectious outcomes to the civilian trauma setting.

1.2.5 Prophylactic antibiotic use after trauma

Antibiotic prophylaxis prior to elective surgery is well established and is reported to significantly decrease surgical site and other post-operative infections (122, 123). Yet there is a paucity of level one evidence to support the practice following major trauma (94, 124). Historically, prophylactic antibiotic guidance for trauma patients has focussed on penetrating abdominal injuries (125, 126) and open fractures (127, 128). More recently, findings from a systematic review supported the use of prophylaxis for penetrating thoracic trauma (129), and this illustrates that much of the available evidence focuses on single system injuries (92, 130). Currently there is a lack of prophylaxis guidelines for patients following multiple severe trauma. Furthermore, a major challenge of exposing patients to antibiotics is the emergence of drug-resistant bacterial strains (131). Gram negative pathogens resistant to broad spectrum antibiotics continue to increase (131, 132). At the same time there is a lack of new

antibiotics being developed, therefore the availability of treatment options is narrowing (133). As a result, current evidence suggests that early but time-limited antibiotic prophylaxis is needed to both decrease post-traumatic infections and minimise resistance (132, 134).

There is an ongoing debate about the duration of antibiotic prophylaxis following trauma. Historical (125, 135) and more recent evidence (92, 124) has debated the appropriate timing of prophylactic antibiotics in trauma patients. This literature reports that prophylaxis for more than 24 hours after injury does not offer enhanced protection against infection, and may increase susceptibility to resistance. A recent systematic review suggested that the risk of bacterial resistance caused by antibiotic administration for 48 hours or more after trauma outweighed potential benefits(132). This conclusion was based on what was described as 'uncertain evidence' and illustrates the lack of quality research trials and clinical consensus in this area of trauma management.

1.2.6 Outcomes following trauma infections

Research specifically investigating patient outcomes as a consequence of post-traumatic infection predominantly focuses on mortality, length of stay and cost. Mortality rates for those who developed infection after injury range from 2-29%. A large retrospective review reported that patients with infection who subsequently developed sepsis had nearly a six-fold greater risk of death compared to those without (Odds Ratio: 5.7, $p<0.001$) (136). Conversely, an earlier large database review of trauma patients found no difference in mortality for those with or without infection (83). However, studies using mortality as a primary outcome varied widely in their methods and patient populations and assessment of outcome rendering them difficult to compare.

There are a number of variables which affect length of stay following trauma, and infection is reported to have an adverse effect on this outcome measure. One study reported increased hospital stays for patients with infection (83). Only 2% of non-

infected individuals were still in hospital at day 15 following injury, compared to 16% of those suffering infectious complications. In research evaluating patients admitted to hospital following road traffic collision, the presence of spinal cord injury was strongly associated with the development of infection (85). Outcome evaluation in this cohort revealed an almost three-fold increase in average length of stay for those suffering infection (No infection: 13 days, vs. Infection: 33 days, $p<0.001$). The availability of specialist or rehabilitation services for spinal cord injured patient may also influence length of stay, however the effects of this were not included in the analysis.

Only one study specifically reported the impact of in-hospital infection on post discharge status (74). Long term outcome analysis revealed worse physical function for up to a year post injury. Patients who had experienced infection reported delayed return to pre injury employment and increased readmission rates when compared to those without infection (74).

Mortality and length of stay are important measures of outcome, yet the development of infection may also have a significant impact on the patients clinical experience in hospital. Published reports do not fully reflect the effect of infection on other outcomes such as organ failure, cardiac injury or venous thromboembolism. A strong association between infection and other outcomes may provide sufficient evidence for this to become a surrogate outcome in future trauma clinical trials.

1.3 Physiological Responses to Trauma

1.3.1 The immune cell response and traumatic injury

The immune system is divided into innate and adaptive responses. The innate immune system is the first level of host defence against infection (137). It is an inherited, evolved

defence mechanism which provides an immediate, non-specific response to harmful stimuli (138). The adaptive immune system is acquired during a lifetime, creating an immunological memory in order to respond to subsequent harmful pathogens (137). The adaptive response is recruited by the innate immune system to supply antibodies to attack specific pathogens (138). Evidence suggests that traumatic injury precipitates a state of immune-suppression which puts patients at risk of pathogen invasion (139, 140). The immune system response to trauma involves a number of different white blood cells (leukocytes). These include granulocytes, primarily neutrophils and monocytes, and agranulocytes, namely lymphocytes.

1.3.1.i Neutrophils

Neutrophils represent between 50 and 60% of all circulating leukocytes and together with monocytes, are the first cells rapidly mobilised in the inflammatory response to trauma (141, 142). Neutrophil numbers are significantly higher in the initial phase following injury (143), recruited by the proinflammatory cytokine response (144). They contribute to a natural immunological defence against microorganisms within minutes of the initial insult (145), lasting up to 72 hours (142). Neutrophils have been implicated in the development of adverse events following trauma. Prolonged inflammation resulting in neutrophil sequestration has been associated with tissue damage leading to the development of MOF (146). Conversely, in patients who sustained a single penetrating injury necessitating operative intervention, early post-operative neutrophil counts demonstrated that neutrophil apoptosis was inversely related to the development of infection (147). In this specific cohort reduced circulating neutrophils resulted in a dampened inflammatory response, thereby limiting tissue injury and infection.

1.3.1.ii Monocytes

Monocytes, together with neutrophils, are fundamental to the innate immune response following injury. They have both pro and anti-inflammatory actions (148). The main

functions of monocytes are phagocytosis and production of cytokines. However, they also play a central role in antigen presentation for recruiting the adaptive immune response (140), namely T-lymphocytes. Severe injury has been associated with alterations in monocyte function resulting in decreased cytokine production (149). Furthermore, monocyte dysfunction frequently co-exists with lymphocyte apoptosis following traumatic injury, and is associated with an increased risk of MOF, acute respiratory distress syndrome (ARDS) and sepsis (150).

1.3.1.iii Lymphocytes

Lymphocytes were traditionally thought to be solely part of the adaptive immune response, however, T-lymphocytes have been shown to also contribute to innate immunity (143, 151, 152). Lymphocytes play an important role in the modulation of inflammation after injury and thus have the potential to affect patient outcome. In the early phases following injury, immunosuppression characterised by lowered lymphocyte counts has been observed (144). Historic evidence suggests that T cell apoptosis was thought to be responsible for declining lymphocyte function following trauma (153, 154) but the exact mechanism for this was unclear. Post injury immunosuppression was previously attributed to an anti-inflammatory response, known as compensatory anti-inflammatory response syndrome (CARS) (144, 155). This was thought to occur after the SIRS response, in order to reduce or 're-balance' the inflammatory state (156, 157) . However, recent genomic studies have questioned this temporal relationship, and suggest both processes occur simultaneously at an early time point following severe injury (152, 158, 159).

The exact mechanism of how lymphocyte counts may be lowered following trauma and the effects of this on outcome are not well described. Anti-inflammatory processes are required to limit the inflammatory response to severe injury, yet a protracted period of immune suppression has been reported to be harmful. Historic evidence suggested decreases in lymphocyte responsiveness lasting up to three days from admission

predisposed critically unwell patients to sepsis (160). In a small group of blunt trauma patients (n=17), depressed lymphocyte responses were associated with the development of MOF and severe sepsis (161). Early, prolonged activation of lymphocyte responses after trauma followed by cellular 'exhaustion', where counts fail to return to normal within three to four days post injury was strongly predictive of mortality (153, 162). Whether lymphocyte count changes after trauma predict the development of infection is unclear.

1.3.2 Inflammation and trauma

The acute inflammatory response to trauma is a complex process which starts early following injury (163). The level of inflammatory response correlates with the severity of injury and is associated with increased mortality and morbidity (164). Inflammation is driven by a number of immune response pathways and inflammatory mediators which are activated after injury (141). Trauma results in an initial state of sterile inflammation which occurs without microbial contamination (165, 166). This inflammatory process is thought have a similar mechanism to the immune system response to bacteria, except the stimulus is tissue damage (167). Following traumatic injury, endogenous 'danger signals' are released from necrotic or dying cells (168). In response, innate immune cell populations are mobilised from the lymph nodes, bone marrow, spleen and tissues. The innate immune cells recognise the so-called 'danger –response' alarm signals (alarmin molecules, also known as danger-associated molecular patterns: DAMPs) (166, 167, 169). The interaction between alarmins, DAMPs and the immune cells activates proinflammatory cytokines, resulting in inflammation (167, 170).

Severe injury can lead to an inflammatory response similar to that seen in sepsis (155, 171). An excessive release of proinflammatory mediators may result in an early, widespread systemic inflammatory response (SIRS) (172). The diagnosis of SIRS is made when at least two of the four clinical parameters seen in Table 1.1 are present.

Table 1.1 Clinical parameters of systemic inflammatory response syndrome (SIRS)

Parameter	Physiological change
Temperature	> 38.8^{C} or < 36.8^{C}
Heart rate	> 90/min
Respiratory rate	> 20/min, or hyperventilation defined as $PaCO_2$ < 32 mmHg
Leukocytes	WBC> 12,000/mm^3 or < 4000/mm^3 Or, the presence of >10% immature neutrophils

Source: Bone et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis; 2009

The degree of SIRS is dependent both on endogenous factors such as metabolic acidosis, and exogenous factors such as the extent of tissue injury (144). The function of post injury inflammation is primarily physiological, acting as a defence mechanism to limit damage, promote healing and prevent further harm (137, 141). Therefore, mild to moderate SIRS may be considered an appropriate response to trauma. However, a persistent, overwhelming inflammatory response may contribute to the development of multiple organ failure (137, 173, 174). Furthermore, a sustained and severe SIRS response may be predictive of infections (108, 109).

1.3.3 Multiple organ failure after trauma

Multiple organ dysfunction syndrome (MODS), describes organ dysfunction present in the first 48 hours following injury (175). Post resuscitation organ dysfunction is common in severe injury and usually resolves within 48 hours (69). This differs from multiple organ failure (MOF), which is a dysfunctional systemic inflammatory response to haemorrhage and subsequent reperfusion injury (23, 68). MOF is frequently reported in severely injured trauma patients, although it is an adverse outcome in many other clinical conditions (144). Nonetheless, trauma is a heterogeneous disease and MOF as a result of injury varies in time course and outcome (18, 69, 175). The diagnosis of MOF lacks an international gold standard (68), and a number of scoring systems have been

developed including the Sequential Organ Failure Assessment Score (SOFA) (176) and the Denver Score (23). These scores provide simple, objective methods of defining and describing individual and multiple organ failure.

Historic studies observed a bimodal pattern of MOF development characterised by 'early' and 'delayed' organ dysfunction (177) . Recent studies have challenged this pattern and it is now accepted that the onset of MOF has a multimodal distribution (23). Most episodes of MOF peak from 48-72 hours following injury, although MOF may develop at any point later in the clinical course as a response to adverse events such as infection or renal failure (178). Recent reports of persistent inflammation-immunosuppression catabolism syndrome (PICS) suggest that this is a late, non-resolving MOF, which further complicates patient recovery and prolongs critical care stay (158, 159, 179). PICS currently lacks a standard definition however clinical determinants include prolonged immunosuppression (lymphocyte count <0.8 $x10^9$/L), persistent inflammation (C-Reactive Protein [CRP]>150 µg/dl) and a critical care stay of more than ten days (158).

MOF as a result of injury affects between 14 and 40% of trauma patients (22, 23, 178). It has been suggested that MOF-related mortality has decreased over the past decade, possibly as a result of contemporary trauma resuscitation practice and improved critical care (23). Risk factors for the development of MOF have altered since early definitions in 1990s, and current evidence reports that age and administration of blood products are independently associated with an increased risk of organ failure (22, 23). Further, a recent study found a strong association between admission shock and the early development of MOF (at 48-72 hours) in blunt injured patients (178). In this cohort 75% of patients who developed MOF also developed an infection. Of note, infection occurred seven days after the diagnosis of MOF and was therefore not temporally associated with organ failure. Whilst admission shock was revealed to be a potential driver for the development of MOF, the relationship between hypoperfusion and infection was not reported.

In summary, immune cell behaviour and subsequent inflammation processes following severe injury are complex, involving a number of interconnected responses. Early immune cell counts, especially in lymphocytes, have been associated with mortality and MOF after trauma. However evidence of the relationship between immune cell counts and infection following severe injury is limited.

1.3.4 Haemorrhage and coagulation dysfunction after trauma

Uncontrolled haemorrhage following trauma is responsible for up to 40% of early in-hospital deaths (18) and despite modern trauma resuscitation, remains the most common and preventable cause of mortality (58, 180). Traumatic haemorrhage is also associated with morbidity, where uncontrolled hypoperfusion, packed red cell transfusion and low admission platelet counts are reported to predict the development of organ failure (22, 23, 69). Injury related changes to the endothelial lining of the microcirculation occur early, and contribute to the development of trauma induced coagulopathy (181). One in four severely injured patients presents to hospital with significant coagulation dysfunction (21, 182).

Acute traumatic coagulopathy (ATC) is an early, endogenous process characterised by systemic anticoagulation and global fibrinolysis (183). ATC appears to be driven by tissue damage and hypovolaemic shock, mediated through activation of the anticoagulant protein C pathways (184). The state of ATC develops rapidly following injury and presents early in the patients course, prior to or independent of any clinical intervention (185, 186). Patients presenting with ATC are reported to have up to 50% mortality rates and a higher incidence of adverse clinical outcomes such as lung injury, sepsis and multiple organ failure (185). Once activated, protein C is associated with a reduction in clotting factors V and VIII, thus slowing down coagulation and producing negative effects on clot strength (187). Inhibition of fibrin formation and increased fibrinolysis are also a key component of ATC (188). Following injury, tissue plasminogen activator (tPA) is slowly released from the endothelium (189), converting plasminogen to plasmin, resulting in fibrinolysis. Following activation of protein C in ATC,

Plasminogen Activator Inhibitor-1 (PAI-1), a protein which normally inhibits tPA is depleted (190). This leads to a de-repression of fibrinolytic activity, resulting in global or 'hyper' fibrinolysis (183, 191). In trauma patients, hyperfibrinolysis is strongly associated with worsening coagulation and increased mortality (188, 192).

1.3.5 Management of trauma haemorrhage and coagulopathy

Initial management of trauma haemorrhage includes administration of crystalloids, red blood cells, plasma, and platelets (58, 193). However, haemorrhage related dysfunction of the microcirculation and subsequent coagulopathy cannot be corrected by volume replacement alone (194). Fluid resuscitation may result in persistent coagulopathy, further bleeding, and poor outcomes (195). Whilst evidence for optimal management and therapeutic targets is lacking at the current time, early haemorrhage control combined with correction of coagulopathy are most likely to reduce the impact of coagulation dysfunction and improve outcomes for bleeding patients (196). Contemporary haemorrhage control techniques range from application of prehospital limb tourniquets (59, 197), haemostatic dressings (198) or balloon occlusion of the aorta (57), to early angioembolisation of bleeding vessels (196) or emergency damage control surgery (193, 199).

Haemostatic resuscitation (HR) used in conjunction with haemorrhage control was developed to assist in the management of trauma induced coagulopathy (200). HR involves targeted blood component transfusion including platelets, plasma and cryoprecipitate, delivered as part of a major haemorrhage protocol (196, 201). However evidence to support the use of HR in the treatment of coagulopathy is limited. Higher plasma to platelet ratios early in resuscitation have been associated with decreased mortality during the first 24 hours of admission, yet after this time point plasma and platelet ratios showed no mortality benefits (58). Further, a recent study observed that correction of coagulopathy does not occur until haemorrhage control is achieved, regardless of blood component ratios (202).

ATC is also characterised by global fibrinolysis. Recent studies have reported rates of admission hyperfibrinolysis to be between 25- 66% of severely injured patients (188, 192). Tranexamic acid (TXA) is an anti-fibrinolytic, anti-inflammatory drug which occupies lysine binding sites on plasminogen, thereby inhibiting plasmin and limiting clot breakdown (186, 191). In a single large multicentre randomized control trial (64) TXA, when given within three hours of injury, demonstrated survival benefits in trauma patients known or suspected to be bleeding. Similar mortality benefits were seen for combat zone patients requiring massive transfusion, where TXA also resulted in an improved coagulation profile (203). These findings have led many services to include TXA in their major hemorrhage protocols (193, 204). Whilst the exact mechanism of action of TXA in bleeding trauma patients is unclear (205) its use is a safe and inexpensive step towards improving the management of haemorrhage and coagulopathy (191). TXA also has anti-inflammatory properties. Plasminogen binds to receptors of cells involved in the development of inflammation process, such as monocytes, neutrophils, and platelets (206). By blocking the binding to these receptors, TXA may modulate the inflammatory response and susceptibility to infection.

Uncontrolled haemorrhage following trauma is a significant cause of mortality and morbidity. Coagulopathy caused by bleeding and tissue damage occurs early after injury and challenges clinical management. Additionally, traumatic haemorrhage and resuscitation induces a cellular ischaemia-reperfusion injury which leads to the activation of an inflammatory response (187, 196). This interplay between the coagulation and inflammatory systems following trauma may further affect outcomes for severely injured patients (181), and be implicated in the development of infection.

Table 1.2 Summary of civilian trauma studies reporting risks and outcomes of infection

Study	Design	Trauma Population	Primary Variable	Infection Rate	Principle Site of Infection	Time to infection	Risk Factors for Infection	Outcomes
Caplan 1981	Retrospective registry review	All trauma patients	Infection	16%	Bacteraemia (21%)	N/S	Invasive devices	Mortality (14%)
Caplan 1985	Retrospective registry review	All trauma patients	Infection	17%	Vascular access sites (21%)	N/S	Invasive devices	Antibiotic use
Pories 1991	Retrospective registry review	All trauma patients	Infection	9%	Urinary tract infection (61%)	N/S	Age, spinal and thoracic injury, surgery	Length of stay (LOS)
Papia 1999	Prospective cohort	All trauma patients	Infection	37%	Respiratory tract (28%)	23 days	Ventilation, surgery, blood transfusion	Mortality (2%)
Bochicchio 2001	Prospective cohort	All trauma patients	Age > 65 years	39%	Respiratory tract (49%)	N/S	Chronic obstructive pulmonary disease	Infection, Mortality (28%), LOS
Bochicchio 2001	Prospective cohort	Blunt injured patients	SIRS on admission	31%	Respiratory tract (38%)	N/S	Admission hypothermia and leukocytosis	Infection, Mortality (8%), LOS
Bochicchio 2002	Prospective cohort	All trauma ICU admissions	Persistent SIRS	41%	Respiratory tract (33%)	8 days	SIRS persisting to day 7 post injury	Mortality (12%), Ventilator days, LOS
Offner 2002	Retrospective database review	Trauma ICU ISS>15	Blood transfusion	40%	Respiratory tract (61%)	7 days	Transfusion of blood >14 & 21 days old	Infection
Lazarus 2005	Retrospective registry review	All trauma patients	Infection	9%	Respiratory tract (42%)	N/S	SIRS, surgery, invasive devices	Mortality (8%), LOS, Costs
Hoover 2006	Prospective cohort	All trauma ICU admissions	SIRS up to day 21	45%	Respiratory tract (22%)	8 days	Admission and persistent SIRS	Infection, Mortality (14%)

Lazarus 2007	Retrospective registry review	All trauma patients	Infection	9%	Respiratory tract (42%)	N/S	Age, ISS, multiple injuries	Infection only
Giamberardino 2007	Retrospective registry review	All trauma patients	Infection	45%	Respiratory tract (49%)	6 days	Multiple injuries, ventilation, surgery, invasive devices	Mortality (29%)
Czaja 2009	Retrospective database review	Trauma patients aged 18-84	Infection	14%	Respiratory tract (57%)	N/S	Male<65 years, obesity, ISS, blood transfusion	Mortality ≤1 year, Functional outcome, Return to work, Re-admission
Sadjadi 2009	Retrospective study	All trauma patients	Blood transfusion	51%	Respiratory tract (61%)	N/S	Blood transfusion	Infection
Yun 2010	Retrospective registry review	All trauma patients	Infection in burns and trauma	8%	Respiratory tract (8%)	7 days	Burns, age, ISS, GCS, comorbidities	Mortality (8%), ICU and hospital LOS
Serrano 2010	Retrospective database review	All trauma patients	Obesity	8%	Respiratory tract (28%)	N/S	BMI, age, ISS, ICU, comorbidities	Ventilator days, ICU and hospital LOS
Bochicchio 2010	Prospective cohort	All trauma ICU admissions	Blood glucose elevation	28%	Urinary tract (16%)	< 14 days	Glucose instability, age, ISS, obesity, diabetes mellitus	Mortality (26%), ventilator days, ICU and hospital LOS
Glance 2011	Retrospective database review	All trauma patients	Infection	5%	Respiratory tract (2%)	N/S	Mechanism of injury, head and chest injuries	Mortality (ICD10 coding), LOS, costs
Fraser 2011	Retrospective registry review	Vehicular trauma	Infection	6%	Urinary tract (11%)	N/S	Age, spinal cord injuries	ICU and hospital LOS, costs
Juffermans 2012	Retrospective database review	All trauma ICU admissions	Blood transfusion	29%	Respiratory tract (46%)	N/S	Transfusion of blood >14 days old	Infection, Mortality (4%), ventilator days

1.4 Project objective and aims

The overall research objective was to characterise factors predictive of infection in severely injured patients. Specifically, the project aims were:

Aim 1. To describe the burden of infections associated with severe injury.

Aim 2. To determine the admission characteristics of severely injured patients who develop infection.

Aim 3. To evaluate the relationship between early immune cell changes after injury and the subsequent development of infection.

Aim 4. To establish whether there is an association between changes in the coagulation system following injury and the subsequent development of infection.

Aim 5. To evaluate the early use of antifibrinolytics on the development of infection following injury.

CHAPTER TWO METHODS

2.1 Study design and setting

This research was a prospective observational study of severely injured patients admitted to a Major Trauma Centre between September 2010 and September 2013. Patients recruited into this research were enrolled into one of two ongoing observational studies within the Centre for Trauma Sciences: Prospective Outcome Evaluation following Trauma (POET) or Activation of Inflammation and Coagulation in Trauma (ACIT II). The POET study was devised and conducted by myself and Karen Hoffman (Research Occupational Therapist). The aims were to investigate infection and other adverse outcomes in severely injured patients admitted to critical care (Elaine Cole) and post discharge (Karen Hoffman). ACIT II is an ongoing study of various aspects of haemorrhage and coagulation dysfunction following traumatic injury (63).

2.1.2 Study participants

All adult trauma patients (>15 years) requiring trauma team activation on arrival and subsequently admitted to the adult critical care unit (ACCU) were enrolled. Criteria for trauma team activation are listed in Table 2.1.

Patients were excluded from ACIT II if there had been a delayed presentation of more than two hours after injury, the administration of over two litres of intravenous fluid prior to Emergency Department (ED) arrival, transfer from another hospital, burns >5% total body surface area, known to be taking anticoagulant medication and those with known bleeding diathesis. For both POET and ACIT II, patients were excluded if they declined to give consent to participate. Finally, severity of injury was calculated using the injury severity score (ISS), and patients with an ISS of less than 16 were excluded retrospectively.

Table 2.1 Trauma team activation criteria

Request by the Helicopter Emergency Medical Service or the London Ambulance Service, or:
History of
Person hit by train
Person trapped under vehicle
Fatality in same vehicle as occupant
Occupant ejected from vehicle
Fall from >2 metres
Presentation with
Amputation proximal to wrist or ankle
Spinal trauma with altered neurology
Chest trauma with altered physiology
Polytrauma with burns
GCS <14 or Respiratory Rate <10 or >29 or Systolic BP <90
Penetrating trauma
Clinical suspicion of
Open or depressed skull fracture
Pelvic fracture
Major haemorrhage

2.2 Consent process

POET was originally reviewed and granted ethical approval by City Road and Whittington Research Ethics Committee in January 2011 (11/LO/1876), and ACIT II by East London and the City Research Ethics Committee 1 in November 2007 (07/Q0603/29).

The majority of severely injured patients enrolled into this study arrived at hospital with an altered conscious level, either as a result of injury or as a consequence of intubation or medication. Those patients who were conscious may have been incapacitated due to pain or psychological distress. As such it may have been inappropriate to discuss trauma research at that time-point and ensure patient comprehension. For ACIT II a

hierarchy of consent was therefore used to recruit patients who were unable to give consent prior to the collection of the research blood samples taken in the ED. Trauma team leaders (ED Consultants), all of whom were independent to the study, acted as the patients advocate and were thus appointed as their Professional Legally Appointed Representative (pLAR). If consent was granted by the pLAR, continued participation was sought from the patient as a priority. Until such time as the patient had capacity, next of kin acted as the consultee, and were asked to consider the wishes of the patient.

Patients and consultees were provided with detailed information sheets and time to consider involvement in POET and ACIT II. If consent was refused or retracted by either the patient or consultee, then participation was withdrawn. In ACIT II, if a patient died before personally giving consent, the consultee was approached for permission to continue the use of samples and patient data for the study. Access to medical notes, pathology test results and patient follow up interviews were required for POET, therefore an invitation to participate in the research was given as soon as the patient was awake and able to comprehend the study aims and protocol.

A paper record of all consent procedures and patient reviews were kept locked in a secure cupboard within a locked office. Anonymised, password protected electronic records were stored in a secure research database within the Centre for Trauma Sciences.

2.3 Data collection

Admission data were collected prospectively on patient demographics, time and mechanism of injury, baseline physiology, coagulation and immune cell profiles. Admission arterial blood analysis for base deficit (BD) was performed during the trauma team resuscitation as part of normal care processes. BD was utilised to detect and predict the severity of hypovolaemic shock on admission to the ED (207, 208). Blood

product use, namely packed red blood cells (PRBC), fresh frozen plasma (FFP), cryoprecipitate and platelets in the first 24 hours following admission were recorded. Patients were reviewed daily until discharge or death, to record clinical outcomes.

2.4 Outcomes

2.4.1 Primary outcome

The primary outcome was the development of infection in critical care. Due to a lack of published infection definitions in Europe, infection was defined using the Centre for Disease Control and Prevention (CDC) criteria as a 'localised or systematic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) occurring ≥48 hours post admission' (209). Data on the incidence of infection was collected daily from patient records, critical care charts, electronic laboratory results and clinical evidence. ED guidelines mandate that prophylactic Co-amoxiclav is commenced in all patients with severe injury requiring admission to critical care (unless penicillin allergic, when Clarithromycin is prescribed). Currently, there is no definitive policy on the recommended duration of prophylactic antibiotics for trauma patients admitted to critical care. The antibiotic prophylaxis is discontinued on the decision of either the critical care consultant or microbiologist during daily patient review.

Infection was confirmed by direct observation of purulent exudate, a combination of adverse clinical signs e.g. pyrexia or pulmonary infiltrates on a chest x-ray (not attributed to acute lung injury) and positive microscopy and culture. Following discussion with the critical care consultant microbiologist, criteria for determining specific sites of infection were developed using standards from the CDC, American College of Surgeons (Surgical site infection)(210) and adaptations with local clinical guidelines (Ventilator associated pneumonia) (Barts Health NHS Trust) (Table 2.2). Discrepancies in the diagnosis of an infection were discussed with the consultant microbiologist. Colonisation, meaning the presence of microorganisms on skin, mucous

membranes, in open wounds, or in secretions but not causing adverse clinical signs or symptoms was not diagnosed as infection.

Table 2.2 Post injury infection site criteria

Location	Criteria
Bacteraemia	Recognised pathogen cultured from one or more blood cultures ***or*** Common skin contaminants cultured from two or more blood cultures on two separate occasions within a 48 hour period.
Respiratory infection (*VAP: not all criteria are required for diagnosis of VAP. Review of clinical VAP chart in patient notes performed daily*)	VAP. Consider if: increased inflammatory markers, pyrexia ≥38.^{0}c or <36.^{0}c, Inotrope requirements increased (NA>0.1), new onset purulent sputum, increased ETT secretions, increased Fi02 since previous day, increased PEEP since previous day, new or progressive infiltrates on chest x-ray, alternative source of infection excluded. Confirmed organisms isolated from sputum. Pneumonia/RTI. Confirmed organisms isolated from sputum, ***and*** increase or change in character of sputum with increased suction requirements. Other criteria to support the diagnosis: pyrexia ≥38^{0}c, ***and/or*** WBC <4,000 or >12,000mm^3/increased inflammatory markers, ***and/or*** worsening gaseous exchange, ***and/or*** new or progressive infiltrates on chest x-ray.
Urinary tract infection	Positive urine culture (collected using appropriate sterile technique), ***and*** elevated WBC count and inflammatory markers. Other criteria to support the diagnosis: pyrexia ≥38^{0}c, ***and/or*** dysuria, ***and/or*** abdominal/flank pain (in conscious patients).
Vascular catheter line infection	Pathogenic organisms cultured from site swab or catheter tip. Other criteria to support the diagnosis: signs and symptoms of phlebitis ***and/or*** purulent discharge from line site ***and/or*** pyrexia ≥38^{0}c.
Traumatic wound infection	**At least one of the following**: Organisms isolated from open, accidental wounds, ***or*** Organisms isolated from contaminated wounds, following cleansing and possibly closure but not requiring surgical intervention, ***or*** Organisms isolated from purulent wound drainage. Other criteria to support the diagnosis: pyrexia ≥38^{0}c, ***or*** localised heat, ***or*** erythema, ***or*** tissue swelling ***or*** tenderness.
Surgical site infection (SSI) (incisional/organ space)	Localised heat, ***or*** erythema, ***or*** tissue swelling ***or*** tenderness, ***or*** pyrexia ≥38^{0}c. ***and*** **at least one of the following**: Organisms isolated from wound, discharge or tissue of surgical incision(s), ***or*** Surgical wound dehiscence ***and/or*** visual evidence of SSI diagnosed by a surgeon.

VAP: Ventilator Associated Pneumonia, NA: Noradrenaline, ETT: Endotracheal Tube, PEEP: Positive End Expiratory Pressure, RTI: Respiratory Tract Infection, WBC: White Blood Cells

2.4.2 Secondary clinical outcomes

The two main secondary outcomes were mortality and organ failure. Mortality included any in-hospital death and was defined as early mortality (≤48 hours) or later mortality (>48 hours). The development of organ failure was determined using the Sequential Organ Failure Assessment (SOFA) score (211). The sum of the maximum score for each organ system was calculated daily (Table 2.3). Scores were measured from 48 hours following admission to exclude multiple organ dysfunction syndrome (MODS), which is common after injury and resuscitation (68, 178). Single organ failure (SOF) was defined as a SOFA score of ≥3 in one organ system during each 24 hour period of the critical care stay. Multiple organ failure (MOF) was defined as SOF in two or more organ systems during each 24 hour period of the critical care stay.

Table 2.3 The Sequential Organ Failure Assessment (SOFA) Score

Organ system	Score 0	Score 1	Score 2	Score 3	Score 4
Respiratory ($Pa0_2/Fi0_2$ ratio: kPa)	>53.3	40-53.3	<40	<25.7	<13.3
Cardiovascular (MAP: mmHg)	≥70	< 70, no inotropes	Dop. ≤5.0 or NE ≤0.05 mcg/kg/min	Dop. 5-14 or NE ≤0.1 mcg/kg/min	Dop. ≥ 14 or NE >0.1 mcg/kg/min
Central Nervous System (GCS)	15	13-14	10-12	6-9	<6
Haematology (Platelets $x10^9/L$)	>150	101-150	51-100	21-50	≤20
Hepatic (Bilirubin µmol/l)	0-19	20-32	33-101	102-204	>204
Renal (Creatinine µmol/l)	<110	110-70	171-299	300-440 or <500ml urine/24H	>440 or <200ml urine/24H

MAP: Mean arterial pressure; Dop: Dopamine; NE: Norepinephrine.

Other clinical outcomes measured included:

- Venous thromboembolism (VTE). The presence of VTE was confirmed by either ultrasound scan (deep vein thrombosis) and/or computed tomography pulmonary angiography (CTPA) (pulmonary embolism).
- Adverse cardiac events, including acute coronary syndrome, supraventricular and ventricular arrhythmias and acute onset heart block. These were confirmed by clinical staff using Troponin T and I levels, ECG and where appropriate echocardiogram.
- Stroke (either ischaemic or haemorrhagic), confirmed by CT scan.
- 28 day ventilator free days.
- Critical care length of stay length of stay (LOS) and total hospital LOS.

2.5 Blood sampling technique

2.5.1 Immune system

Blood tests were taken to measure immune cell counts and evaluate the relationship with infection (described in full in chapter 4). On admission and whilst in critical care, white blood cell counts (WBC) were measured daily as part of routine inflammatory assessment. Blood samples were collected each morning by clinical staff in an EDTA tube (Beckton Dickinson, Plymouth, UK), for WBC (leukocyte), neutrophil, monocyte and lymphocyte counts. Normal reference ranges are detailed in Table 2.3.

2.5.2 Coagulation system

Blood tests were taken to measure coagulation after injury and evaluate the relationship with infection (described in full in chapter 5). As part of ACIT II, a 30 ml research sample of blood was drawn at admission and 24 hours following injury by the trauma research team. Samples for fibrinogen and coagulation factor assays were

collected into 4.5ml glass vacutainers (0.109 M buffered sodium citrate, 3.2% - Becton Dickinson, Plymouth, UK). Samples for Factor assay, Protein C (PC) and Protein S (PS) were centrifuged with double spun plasma extracted and stored at -80˚C within two hours of venepuncture. Normal reference ranges are detailed in Table 2.4.

Table 2.4 Blood sample normal reference ranges

Test/Factor	Normal range	Test/Factor	Normal range
WBC	4-11 10^9/L	**Factor IX**	58-138 iu/dL
Neutrophils	2.5-7.5 10^9/L	**Factor X**	50-150 iu/dL
Monocytes	0.2-0.8 10^9/L	**Factor XI**	58-148 iu/dL
Lymphocytes	1-4 10^9/L	**Factor XIII**	70-140 iu/dL
PT	9.4-12.4 seconds	**Anti-thrombin**	81-119 iu/dL
APTT	21-31 seconds	**Protein C**	72-162 iu/dL
INR	0.9-1.1 iu/dL	**Protein S**	62-120 iu/dL
Fibrinogen	1.50-4.50 g/L	**PAP**	120-700 µg/L
Factor II	78-117 iu/dL	**a2ap**	76-126 iu/dL
Factor V	66-114 iu/dL	**PAI-1**	4-43 ng/mL
Factor VII	50-150 iu/dL	**tPA**	2–12 ng/mL
Factor VIII	52-153 iu/dL	**D-Dimer**	<550 ng/mL

WBC: White blood cells; PT: Prothrombin time; APTT: Activated partial thromboplastin time; PAP: Plasmin antiplasmin, a2ap: α_2-antiplasmin, PAI-1: Plasminogen activator inhibitor 1, tPA: tissue plasminogen activator, DD: D-dimer.

Blood for rotational thromboelastometry (ROTEM, TEM innovations GmbH, Munich, Germany) viscoelastic coagulation testing was also taken at 24 hours. ROTEM assesses the viscoelastic properties of the blood samples under low shear conditions (212). At 24 hours post injury it was used to measure the clotting time (CT), clot formation time

(alpha angle), clot amplitude at 5 minutes (CA5) and the ultimate strength and stability of the clot (maximum clot firmness [MCF]). This sample was drawn into a 2.7ml citrated vacutainer (0.109 M buffered sodium citrate, 3.2% - Becton Dickinson, Plymouth, UK) and processed in the trauma research laboratory.

2.6 Blood sample analysis

WBC differential testing required an 18µl sample of blood for fully automated analysis using flow cytometry. Samples were processed in the hospital haematology laboratory using a Sysmex SE-2100 blood analyser (Sysmex, Milton Keynes). If more than one WBC test had been performed in a 24 hour period, the most abnormal results were recorded. Normal ranges were defined using the Barts NHS Trust laboratory guidance: Total WBC 4-11 $x10^9$/L, Neutrophils 2.5-7.5 $x10^9$/L, Monocytes 0.2-0.8 $x10^9$/L and Lymphocytes 1-4 $x10^9$/L.

Blood samples for coagulation analysis were processed by the hospital haematology laboratory. This included prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and D-Dimer (DD). Coagulation factors, PC and PS were measured using an automated analyser (Sysmex CA-1500 System, Siemens AG, Germany). Plasma activity levels of α2-antiplasmin were assayed using a Sysmex CS2100i automated analyser (Siemens AG, Germany).

ROTEM was performed within one hour of blood draw at 37°C on a ROTEM delta instrument (Pentapharm GmbH, Munich, Germany) by the ACIT II research team. 20µl of recalcitrant (STARTEM) and 20µl of tissue factor derived from rabbit brain (EXTEM) were placed into the test cuvette after which 300µl of the blood sample was added. Activation with tissue factor was performed to standardize the in vitro coagulation process and produce a more rapid result (63). All pipetting steps and the mixing of reagents with samples were performed as standard using the automated electronic

pipette program. Clotting Time (CT), Clot Amplitude at 5 minutes (CA5), alpha angle (α angle) and maximum clot firmness (MCF) were reported for each sample analysed. The coefficient of variation for each result is presented in Table 2.5.

Table 2.5 ROTEM repeatability and reproducibility (coefficient of variation %)

Precision	CT	Alpha angle	CA5	MCF
Intra-assay	6	1	2	3
Inter-instrument	7-13	2-3	2-3	1-3

2.6.1 Enzyme linked immunosorbant assays (ELISA)

Fibrinolysis is a central component of the coagulation system, and therefore an important part of the investigation in aim four. Specific markers of fibrinolysis not analysed in the hospital laboratory were quantified using Enzyme Linked Immunosorbant Assays (ELISAs). Tissue plasminogen activator (tPA; Asserachrom tPA, Diagnostica Stago, Asinieres, France) was assayed by Claire Rourke, Research Assistant, Centre for Trauma Sciences. Plasmin-α2-antiplasmin complex (PAP; DRG PAP micro, DRG International, Marburg, Germany) and Plasminogen activator inhibitor 1 (PAI-1; DRG PAI-1, DRG International, Marburg, Germany) were assayed by Elaine Cole.

2.6.2 PAP ELISA protocol

1. Reagents were provided in two 96 microtitre wells coated with anti-PAP monoclonal antibody, allowed to come to room temperature prior to use.
2. Plasma samples selected and thawed for five minutes in water bath at 37 ^{0}C
3. 30mL wash solution diluted with 1170 mL de-ionised water
4. 50 µl (microliters) of Assay Buffer dispensed into each well
5. 50 µl of standard/ control/sample added to wells and mixed thoroughly
6. Adhesive cover placed over wells and incubated for 15 minutes at room temperature

7. Wells washed three times with wash solution using plate washer
8. 400 µl of enzyme conjugate diluted with 22mL conjugate diluent
9. 100 µl of enzyme conjugate dispensed into each well
10. Adhesive cover placed over wells and incubated for 15 minutes at room temperature
11. Wells washed three times with wash solution using plate washer
12. 100 µl Tetramethylbenzidine substrate solution added to each well
13. Wells covered with aluminium foil and left at room temperature for 15 minutes
14. 100 µl of stop solution (sulphuric acid) added to each well
15. Absorbance of each well read at 450 nm (nanometre) within 30 minutes of step 14

Calculation of results:

The absorbance of the ELISA data (optical density=OD) at 450 nm was measured using a SIAFR Synergy HT plate reader (BioTek, Winooski, USA). Concentrations of the samples were determined from the standard curve plotted using GraphPad PRISM version 5 (GraphPad Software Inc, San Diego, CA, USA) in accordance with both the manufacturer and GraphPad Prism instruction manual. Mean absorbance was converted to microgram per millilitre (µg/L) using linear regression. A normal median plasma PAP level in healthy adults is 290µg/L. The coefficients of variation for intra-assay reproducibility were 2.1-6.3% and for inter-assay reproducibility were 3.5-11%.

2.6.3 PAI-1 ELISA protocol

1. Reagents were provided in two 96 microtitre wells coated with anti-PAI-1 monoclonal antibody, allowed to come to room temperature prior to use.
2. Plasma samples selected and thawed for five minutes in water bath at 37 ^{0}C
3. 30mL wash solution diluted with 1170 mL de-ionised water
4. 100 µl (microliters) of Assay Buffer dispensed into blank designated wells and 50 µl of Assay Buffer dispensed into sample designated wells

5. 50 µl sample added to sample designated wells
6. 50 µl 1:100 Biotin Conjugate added to all wells and mixed thoroughly
7. Adhesive cover placed over wells and incubated for two hours at room temperature
8. Wells washed three times with wash solution using plate washer
9. 100 µl 1:200 diluted Streptavidin-HRP solution added immediately to all wells
10. Adhesive cover placed over wells and incubated for one hour at room temperature
11. Wells washed three times with wash solution using plate washer
12. 100 µl Tetramethylbenzidine substrate solution dispensed into each well
13. Wells covered with aluminium foil and left at room temperature for 10 minutes
14. 100 µl of stop solution (sulphuric acid) added to each well
15. Absorbance of each well read at 450 nm within 15 minutes of step 14

Calculation of results:

Results were calculated using the same methods described for PAP (above), although mean absorbance was converted to picogram per millilitre (pg/ml) for PAI-1. The normal plasma concentration range of PAI-1 in healthy adults is 4-43 ng/mL (4000-43000 pg/mL). Coefficient of variation is shown in Table 2.6.

Table 2.6 Median PAI-1 concentration and coefficient of variation

Human PAI-1 concentration pg/mL	Coefficient of variation (%)
5000	1.3
2500	1.7
1250	1.6
625	3.0
313	3.7
156	5.0
78	6.4

2.7 Data analysis

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego CA USA). Normal quantile plots were used to test for normal distribution. Parametric data were expressed as means (95% Confidence Intervals) and were analysed using Students t test or ANOVA. Non-parametric data were expressed as medians (Interquartile Range - IQR) and analysed using Mann Whitney U or Kruskal Wallis tests. Categorical variables were analysed using Chi square or Fisher's exact tests. A p value of <0.05 was considered statistically significant.

Multivariate analysis was used to determine statistically independent relationships between infection and other variables, using SPSS v21 (IBM Corporation, Armonk, NY, USA). Multivariate linear regression was used to analyse continuous dependent variables and binary logistic regression for binary dependent variables.

CHAPTER THREE THE BURDEN AND DRIVERS OF INFECTION IN SEVERELY INJURED PATIENTS

3.1 Introduction

Contemporary healthcare has placed great emphasis on infection prevention (209, 213), yet infections continue to have a major impact on patient outcomes and experience. Developing an infection after trauma is associated with increased critical care utilisation and hospital stay (73, 83, 136). Injured patients who survive infection to hospital discharge have reported delayed return to functional, psychological and social recovery (74). Despite this, infection remains an under-reported and under-recognised cause of morbidity in severely injured patients, with much of the evidence derived from retrospective reviews (66, 86).

The true incidence of infection for severely injured patients is difficult to ascertain from the published evidence as many civilian studies include all trauma patients, regardless of acuity or injury severity. Furthermore, studies focus on singular determinants of infection such as the association with blood transfusion (111), mechanism of injury (85, 88) or invasive intervention (99, 214). Therefore the global burden of infection in the severely injured population is not widely appreciated, the drivers for its development remain unclear, and reports of the effects on clinical outcomes are predominantly limited to mortality (67, 86, 108, 109). Overall there is a significant knowledge gap in the incidence, drivers and impact of all infections following severe injury in modern civilian trauma care.

3.2 Study objective and aims

The overall objective of this study was to characterise the burden of all-cause infection in severely injured trauma patients.

The first aim was to describe the incidence of infection in severely injured patients.

Secondly to identify which admission, patient or injury characteristics were associated with the development of infection.

Finally, to evaluate outcomes in those patients who developed an infection following severe injury.

3.3 Methods

This was a prospective cohort study of severely injured patients presenting to an urban major trauma centre over a two year period from February 2011.

3.3.1 Patient selection

Adult patients (>15 years), admitted to the critical care unit following resuscitation and found to have an injury severity score (ISS) of greater than 15, were enrolled. Patient recruitment processes have been described in detail in chapter 2.1.2.

3.3.2 Data collection

Data were collected prospectively on patient demographics, mechanism (blunt or penetrating injury), baseline physiology, coagulation and immune cell profiles. Arterial blood analysis for base deficit (BD) was performed during the trauma team resuscitation on admission as part of normal care processes. BD ≥6mEq/L was utilised to predict the presence of hypovolaemic shock on admission to the ED (207, 208). Blood product use

was recorded, namely Packed Red Blood cells (PRBC) and Fresh Frozen Plasma (FFP), in the first 24 hours following admission. Injury severity was classified using the Injury Severity Score (ISS) once all injuries were diagnosed (29).

3.3.3 Outcome measures

Patients were followed up daily until in hospital death or discharge. The primary outcome measure was the presence of infection developed in critical care. Data on the incidence of infection and use of prophylactic antibiotics was collected daily from patient records, critical care charts, electronic laboratory results and discussion with clinicians. Infection was defined using the Centre for Disease Control and Prevention (CDC) criteria as a 'localised or systematic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) occurring ≥48 hours post admission' (209). Infection was confirmed by direct observation of purulent exudate, adverse clinical signs e.g. pyrexia or pulmonary infiltrates on a chest x-ray (not attributable to lung injury) and positive microscopy and culture. Criteria for determining specific sites of infection are discussed in chapter 2.4.1. Unless penicillin allergic, severely injured patients were given the penicillin combination Co-Amoxiclav for antibiotic prophylaxis on admission to critical care. This was reviewed daily and discontinued on the advice of the consultant microbiologist.

Other outcomes measured were in-hospital mortality ≥48 hours following admission, ventilator free days (VFD), incidence of venous thromboembolism, incidence of adverse cardiac events such as new atrial fibrillation or tachyarrythmias, critical care length of stay (LOS) and total hospital LOS. The development of organ injury was assessed daily using the Sequential Organ Failure Assessment (SOFA) score (211). Single organ failure (SOF) is defined as a SOFA score of ≥3 in one organ system during each 24 hour period of the critical care stay (176) . Multiple organ failure (MOF) is defined as SOF in two or more organ systems during each 24 hour period of the critical care stay.

3.3.4 Data analysis

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego CA USA). Normal quantile plots were used to test for normal distribution. Parametric data were expressed as means (95% Confidence Intervals) and were analysed using Students t test or ANOVA. Non-parametric data were expressed as medians (IQR) and analysed using Mann Whitney U test or Kruskal Wallis. Proportions of dichotomous variables were analysed using Chi square or Fisher's exact tests depending on the size of the sample. A p value of <0.05 was considered statistically significant.

Multivariate analysis using SPSS v21 (IBM Corporation, Armonk, NY, USA) was utilised to determine statistically independent relationships between admission variables and the development of infection. This approach was also used to examine independent relationships between infection and other variables on clinical outcomes. Factors achieving significance of $p<0.15$ in univariate analysis were entered into the regression models. Multivariate linear regression was used to analyse continuous dependent variables and binary logistic regression for binary dependent variables.

3.4 Results

In the 48 month period 385 trauma patients were admitted to the critical care unit. Of these, 300 patients had an ISS >15 and were enrolled into the study. Within the first 48 hours 29 (10%) patients died, leaving a cohort of 271 available for analysis. 141 patients (52%) developed at least one infection during their critical care stay, and there were 304 infections diagnosed overall, which is an average of two infectious episodes per patient.

Gram positive bacteria caused the greatest infection burden (Table 3.1). In blood stream infections, the presence of gram positive microbes was approximately two-fold

higher, with a similar trend seen in vascular access device, traumatic wound and surgical site infections.

Table 3.1 Infections developed during critical care stay

Infection site	Number of Infections n=304	Gram positive bacteria	Gram negative bacteria	Fungal microbes
Respiratory	176 (57%)	42%	50%	8%
Bacteraemia	34 (11%)	62 %	33%	5%
Traumatic wound	45 (15%)	64%	33%	3%
Vascular access device	27 (9%)	64%	32%	4%
Urinary tract	11 (4%)	27%	73%	0
Surgical site infection	11 (4%)	55%	36%	9%

The most prevalent site of infection was the respiratory tract. These infections were five times more common than those found in the blood stream and four times more common than those affecting traumatic wounds (Table 3.1). The first day of infection occurred early in the patients clinical course (Figure 3.1), at day five post injury for the majority of patients.

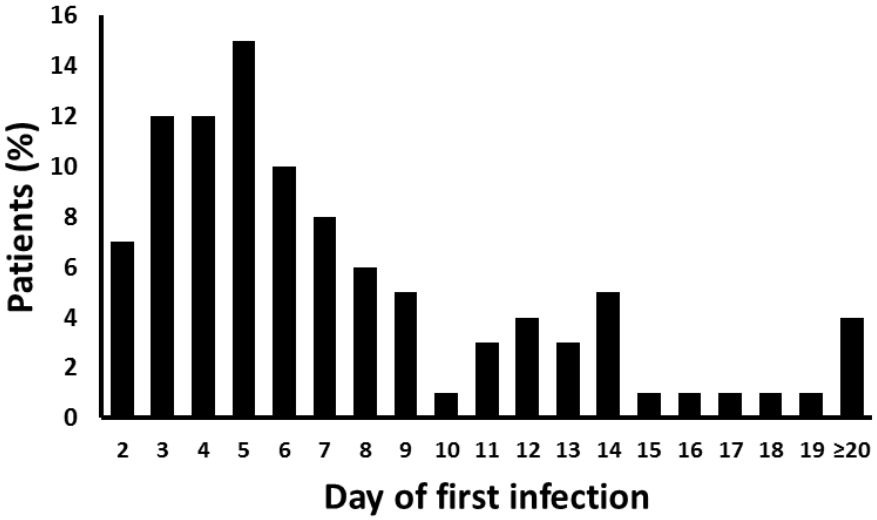


Figure 3.1 Day of first infection post injury. Bar chart shows percentage of patients per day of first infection.

Different infections presented at different periods after injury. Respiratory infections occurred earliest (Figure 3.2), a median of five days from admission. Median time to most other infections was two days later, except SSIs which occurred at median 14 days after admission.

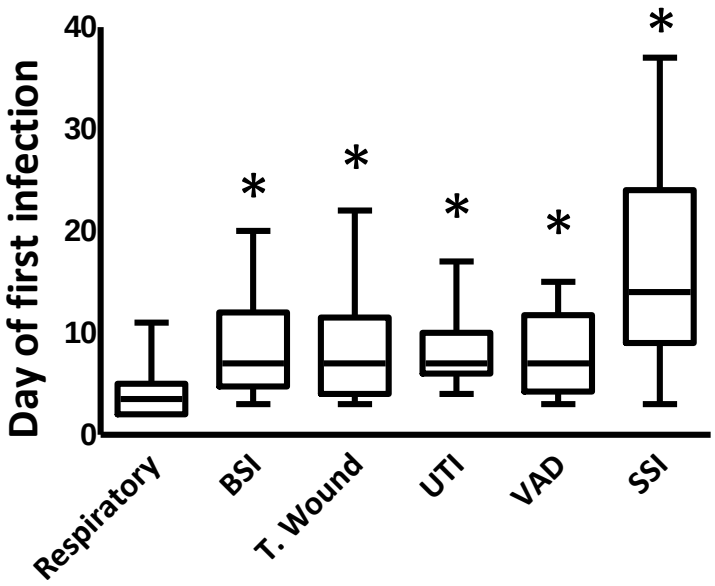


Figure 3.2 Differing infectious loci present at different times. Box and whisker plots show median and interquartile range of respiratory infection at median 5 days vs. blood stream infection at median 7 days $p<0.001$, traumatic wound infection at median 7 days $p<0.001$, urinary tract infection at median 7 days $p=0.01$, vascular access device infection at median 7 days $p=0.03$, surgical site infection at median 14 days $p<0.001$.

Table 3.2 Patient characteristics, injuries and admission physiology

	All n=271	No infection n=130 (48%)	Infection n=141 (52%)	p value
Admission characteristics				
Age†	35 (25-49)	35 (25-28)	35 (26-50)	0.93
Male	223 (83%)	105 (81%)	119 (85%)	0.57
Blunt injury	235 (87%)	111 (85%)	125 (89%)	0.52
Admission injuries				
ISS†	29 (21-36)	26 (20-38)	29 (22-36)	0.14
AIS Head and neck	4 (1-5) [58%]	4 (1-5) [60%]	4 (1-5) [56%]	0.98
AIS Face	0 (0-2) [5%]	0 (0-2) [5%]	0 (0-2) [4%]	0.99
AIS Thorax	3 (1-4) [59%]	3 (1-4) [56%]	3 (0-4) [61%]	0.99
AIS Abdomen & pelvis	0 (0-2) [12%]	0 (0-2) [13%]	0 (0-2) [11%]	0.95
AIS Extremity & pelvic ring	2 (0-3) [31%]	1 (0-3) [27%]	2 (0-3) [34%]	0.37
Admission physiology				
SBP (mmHg)†	122 (89-140)	123 (93-142)	120 (86-139)	0.48
BD (mEq/L) †	3.4 (0.9-7)	2.1 (-0.8-5.8)	4.3 (2.2-8.4)	0.01
GCS†	11 (6-15)	12 (5-15)	11 (7-15)	0.69
APTT (secs) †	25 (23-29)	25 (23-28)	26 (23-29)	0.18
INR†	1 (1.1-1.2)	1 (1.1-1.2)	1 (1-1.3)	0.97
Platelets (units) †	206 (156-252)	201 (149-249)	213 (165-256)	0.53
Fibrinogen (units) †	2 (1.38-2.26)	2 (1.31-2.26)	2 (1.4-2.26)	0.99
PRBC in first 24 hours†	4 (3-4)	3 (2-4)	4 (3-5)	0.01
FFP in first 24 hours†	3 (2-3)	2 (1-2)	3 (2-4)	0.02
Prophylactic antibiotics	239 (88%)	110 (85%)	129 (91%)	0.27
Duration of prophylactic antibiotics (days) †	3 (2-3)	2 (2-3)	3 (2.3)	0.31

Values are presented as median (inter quartile range)† or number (%). Values in [square brackets] are % AIS ≥3. ISS: Injury Severity Score; AIS: Abbreviated Injury Scale; BD: Base Deficit; SBP: Systolic Blood Pressure; GCS: Glasgow Coma Scale; APTT: Activated Partial Thromboplastin Time; INR: International Normalised Ratio; PRBC: Packed red blood cells; FFP: Fresh frozen plasma.

Evaluation of admission characteristics revealed that there were no significant differences in age, gender, mechanism or severity of injury and prophylactic antibiotic use between patients who developed infection, and those who did not (Table 3.2). However, severity of shock on admission to hospital was significantly greater in patients who subsequently suffered infection (No infection: BD 2.1 mEq/L vs. Infection: BD 4.3 mEq/L, p=0.01). Similarly, increased blood product requirements in the first 24 hours from injury were associated with infection (Table 3.2).

Rates of infection were greater in those who were shocked on admission. A dose dependent relationship was seen between severity of shock and increased percentage of infection (Figure 3.3). This increased to two fold higher in the most severely shocked cohort.

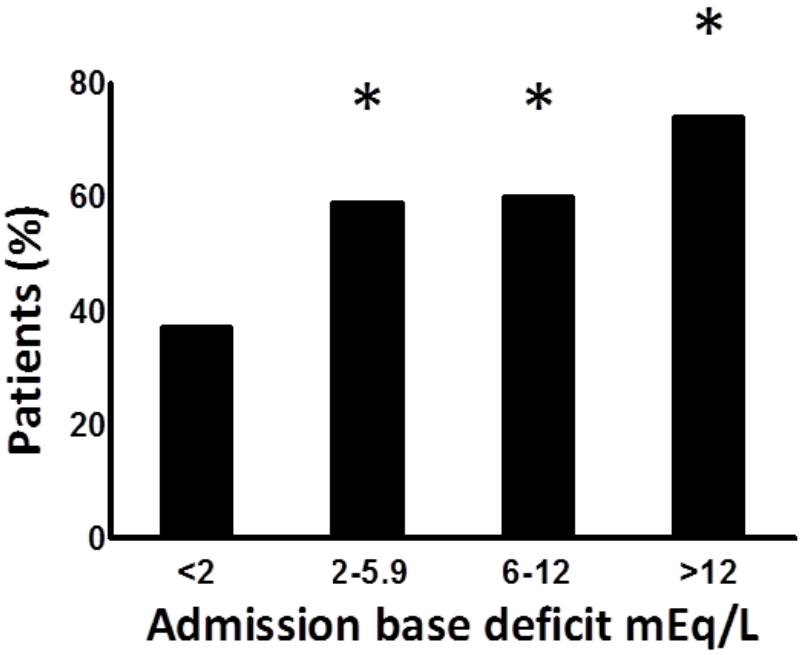


Figure 3.3 The development of infection is associated with the degree of admission shock. Graph shows the percentage of patients with infection in admission base deficit quartiles (mEq/L). Shocked patients were significantly more likely to develop infection than non-shocked patients (BD 2-5.9 mEq/L vs. normal BD<2 mEq/L - 59% vs. 37%, p<0.01; BD 6-12 mEq/L vs. normal BD<2 mEq/L - 60% vs. 37%, p<0.01; BD>12 mEq/L vs. normal BD<2 mEq/L - 74% vs. 37%, p<0.01).

The time to first infection was also related to shock. Median time to first infection was earlier for those in shock compared to those patients not shocked on admission. However the most severely shocked patients developed their first infection earliest, on average two days prior to the non-shocked cohort (Figure 3.4).

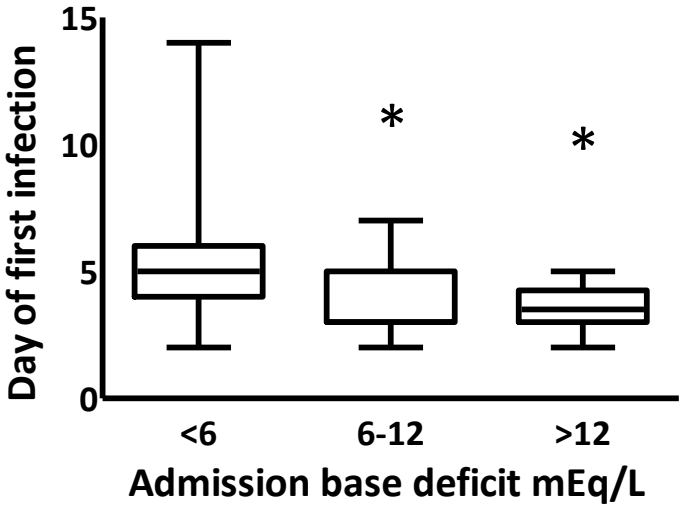


Figure 3.4 Increased shock severity is associated with the timing of infection. Box and whisker plots show median and interquartile range of day of first infection according to admission base deficit (mEq/L). Median time to first infection was earlier for all degrees of shock compared to those patients not shocked on admission. Shocked patients were significantly more likely to develop infection earlier than non-shocked patients (BD 6-12 mEq/L: 3 days (IQR 3-5) vs. normal BD <6mEq/L: 5 days (IQR 4-7), $p<0.01$; BD>12 mEq/L: 3 days (IQR 3-4) vs. normal BD<2 mEq/L: 5 days (IQR 4-7), $p<0.01$).

In order to determine an independent relationship between potentially predictive admission variables and the development of infection, multivariate logistic regression was performed. Factors achieving significance of $p<0.15$ in univariate analysis were entered into the regression model. Independent variables with univariate significance were ISS, BD, PRBC and FFP. After adjusting for the effects of these variables, regression analysis showed that only admission BD ($p<0.001$, OR 1.78, CI 1.48-1.94), was independently associated with the development of infection ($R^2 = 0.63$). Therefore, shock severity was the only admission variable independently driving the development of infection in this cohort of severely injured patients.

No differences were seen in unadjusted mortality rates for both groups (9%). However incidence of multiple organ failure (MOF) during the critical care stay was significantly increased in the infection group compared to those without infection (Infection: MOF - 35% vs. No infection: MOF - 8%, p<0.01) (Table 3.3).

Table 3.3 Outcomes after infection

	All n=271	No infection n=130 (48%)	Infection n=141 (52%)	p value
Mortality ≥48H	25 (9%)	12 (9%)	13 (9%)	0.96
Multiple organ failure	60 (22%)	11 (8%)	49 (35%)	<0.01
VTE	7 (3%)	1 (1%)	6 (4%)	0.40
Adverse cardiac event	49 (19%)	12 (9%)	37 (26%)	0.05
VFD†	25 (18-27)	27 (26-28)	19 (12-23)	<0.01
CC LOS†	6 (2-12)	2 (2-12)	12 (8-20)	<0.01
TH LOS†	22 (8-41)	8 (3-17)	33 (25-53)	<0.01

Values are presented as median (inter quartile range) † or number (%). VFD: Ventilator Free Days; VTE: Venous thromboembolism; CC LOS: Critical care length of stay; TH LOS: Total hospital length of stay

There was trend to significance for patients with infection and the development of adverse cardiac events (Table 3.3). Further, patients who developed infection had significantly fewer ventilator-free days compared to those who did not (Infection: 19 days vs. No infection: 27 days, p<0.01). In total, patients with infection spent 10 days longer in critical care (Infection: 12 days vs. No infection: 2 days, p<0.01) and 25 days longer in hospital compared to those without infection (Infection: 33 days, vs. No infection: 8 days, p<0.01).

To establish if infection or other variables had an independent effect on outcomes other than mortality, multivariate analysis was performed. Factors achieving p<0.15 during

univariate analysis were entered into a multivariate regression analysis (Table 3.4). In order to find the best statistical correlation between variable and outcome in multivariable linear regression, stepwise analysis was chosen with entry at 0.05 and removal at 0.10.

Table 3.4 Univariate and multivariate regression analysis of variables associated with outcome

	Univariate analysis		**Multivariate analysis**		
Significant variable	**p value**	**Odds ratio/ Beta co-efficient**	**95% Confidence Intervals**	**p value**	**R sq**
MOF					
Infection	<0.01	15.4	8.20-28.9	<0.01*	0.40
PRBC	0.13	1.03	0.95 – 1.07	0.65	
VTE					
PRBC	0.09	1.00	0.87 – 1.15	0.91	0.27
Infection	0.05	1.24	0.74 – 5.25	0.09	
VFD					
PRBC	0.14	-0.17	-0.39 – 0.09	0.15	0.52
Infection	0.10	-4.48	-6.76 - -2.13	<0.01*	
CCLOS					
Age	0.04	0.41	0.13 – 0.61	0.24	0.45
ISS	0.05	0.14	-0.01 – 0.30	0.08	
Infection	0.05	13.2	10.0 – 16.4	<0.01*	
THLOS					
ISS	0.01	0.33	0.04 – 0.65	0.04*	0.47
Infection	0.01	31.1	24.0 – 38.2	<0.01*	

MOF: multiple organ failure, PRBC: packed red blood cells, VTE: venous thromboembolism, VFD: ventilator free days, CCLOS: critical care length of stay, ISS: injury severity score, THLOS: total hospital length of stay.

Infection was the only factor independently associated with MOF ($p<0.01$), days on a ventilator ($p<0.01$) and critical care LOS ($p<0.01$). Both infection ($p<0.01$) and injury severity ($p=0.04$) had a significant independent relationship with total hospital stay.

After adjusting for potentially confounding variables, the development of infection was significantly associated with poor clinical outcomes.

3.5 Discussion

Infection is a substantial burden for severely injured civilian patients admitted to critical care. Infection in this cohort was common, with over half of the patients developing an infectious episode. Similar to other studies of trauma infections, the respiratory tract was most commonly affected; however the majority of infections occurred earlier than had previously been appreciated. Shock was the only admission characteristic associated with infection in critical care. Infection also developed more quickly in patients who presented in shock. The development of infection was associated with significantly worse outcomes for severely injured trauma patients.

In this cohort of civilian trauma patients, 52% suffered at least one infection following injury, which is higher than recently published prospective studies (67, 109). Historic prospective evidence reported a 37% incidence of infection (71), although severity of injury or physiological status on arrival was not analysed. Early or persistent SIRS responses following injury have been associated with infection rates between 41 and 45% (108, 109). More recent military studies reported infection rates of up to 50% in blast injured patients (87, 119), with a mechanism of injury that obviously differs from patient in this study.

Whilst the respiratory tract was most commonly affected, the location and types of infection developed in this cohort were heterogeneous. Severe infection such as ventilator associated pneumonia or bacteraemia may be more clinically significant when compared to relatively simple infections such as those affecting the urinary tract. However trauma is itself a heterogeneous disease and physiological disturbances as a result of injury may lead to an increased risk of infection which would have fewer

consequences in a non-injured patient. For example, whilst relatively innocuous for many, infections of the urinary tract are associated with a greater mortality following traumatic injury (215). Furthermore due to the number of invasive interventions required, managing trauma patients in critical care provides an added risk for infectious complications (216). All episodes of confirmed infection, regardless of the location or bacteria, have the potential to alter the trauma patients clinical course whilst in critical care. In this study infections resulted in prolonged treatment in critical care, and the presence of an infection was strongly associated with increased ventilator dependence and the development of multiple organ failure. Other studies of infection in trauma patients have also reported increased hospital stays (83), increased healthcare costs (73, 136) and delayed longer term recovery (74).

Infection was not only a great burden, but also occurred earlier than expected. Historic studies reported infections occurring two to three weeks post hospitalisation (70, 71). However, more recent comparable evidence observed first infections occurring at day eight post injury (67, 108, 109). In this study, the greatest numbers of first infections were diagnosed at day five post injury, suggesting that either the injury or an early admission factor may be associated with the development.

The identification of admission shock as the sole driver of infection was surprising. No other patient or injury characteristic such as age, gender or mechanism was statistically associated with infection in this severely injured cohort. Previous studies that have associated injury severity with infection did not include assessments of shock severity in the analysis (24, 66, 111, 136). The severity of shock also correlated with the timing of infection. For those patients who were profoundly shocked on arrival (BD>12 mEq/L), infections occurred even earlier, on average at three days from injury.

Haemorrhagic shock may activate pathways that predispose patients to infection. There is a high degree of crosstalk between coagulation and inflammation, and early

coagulopathy has been associated with the subsequent development of VAP (96, 217) . Tissue injury and trauma-related hemorrhage stimulate an inflammatory response initially characterised by increased levels of proinflammatory cytokines and activation of neutrophils and monocytes (147). However severe trauma is also associated with immunosuppression, predominantly seen as anergy in the adaptive immune system T-lymphocyte populations (144, 152). Therefore, injury related immunosuppression may lead to increased susceptibility to infections. Blood transfusion has also been associated with increased infectious episodes (97, 111, 218, 219). While transfusion may have effects on immunocompetence, it is possible that these are simply a reflection of the underlying degree of shock. Admission shock was the driver for infection in this study and improved early shock management may lead to a reduction in the overall burden of infection.

The consequences of infection following injury and the impact these have on patient outcome and experience are underappreciated in relation to morbidity. Patients who developed infection had significantly worse outcomes than those who did not. While there were patient and injury differences, infection was an independent predictor of multiple organ failure, ventilator-free days, critical care and hospital stays. Establishing the cause of trauma related infections may help to focus on prevention, and have a considerable impact on outcome and patient experience.

This study has a number of limitations. Principally the associative relationship found between admission shock and infection, where a potential driver rather than a cause was identified. However shock was found to independently predict infection in multivariate analysis, and this suggests that the inflammatory and coagulation processes which occur early following injury may be implicated. A further limitation is that outcomes between the two groups following discharge from hospital were not measured. The effects of infection developed in critical care following injury may be far reaching. Understanding longer term physical and psychological consequences of infection may be important patient and system performance outcome measures.

3.6 Conclusion

The study has shown that infectious complications are a significant burden for severely injured patients. Infections occur early in the critical care stay and are associated with worsened clinical outcomes. Severity of admission shock was predictive of infection. This represents an opportunity for further study into the inflammatory mechanisms linked to haemorrhage, and investigation into their relationship with infection.

CHAPTER FOUR CHANGES IN IMMUNE CELL COUNTS AFTER SEVERE INJURY AND THE RELATIONSHIP WITH INFECTION

4.1 Introduction

Current concepts suggest that the proinflammatory state after trauma is driven by immune cell activation, primarily neutrophils and monocytes (163, 174). A simultaneous anti-inflammatory response occurs where neutrophils, monocytes and lymphocytes attempt to 're-balance' the pro-inflammatory state (148, 152, 220). Specific evaluation of immune cell counts and their relationship with infection in trauma patients is limited. The few papers which have reported white cell counts and their effects on outcome after injury have shown associations with mortality (108, 153, 162) and MOF (161). This evidence suggests that changes to immune cell counts in the days following trauma are associated with adverse outcome.

Severe injury may result in suppression or alteration of immune cell responses which may predict the development of infection. The research presented in chapter three showed that infection after trauma developed in the first week following injury and was strongly associated with admission shock. The proposed relationship between haemorrhage and inflammation has been previously described however it is unclear if hypoperfusion affects immune cell counts following severe injury. Furthermore, changes in immune cell counts related to trauma may be predictive of the development of infection, and may help in the identification of at risk patients.

4.2 Study objective and aims

The overall objective of this study was to evaluate early immune cell counts following injury and the subsequent development of infection in trauma patients.

First, to evaluate the effect of admission hypoperfusion on early leukocyte (WBC), neutrophil, monocyte and lymphocyte counts following severe injury.

Second, to determine if early changes in immune cell counts following severe injury are predictive of infection.

Third, if a predictive relationship is found, to identify an association with the timing and site of injury related infection.

Finally, if early immune cell counts are found to be predictive of infection, to determine if they are also predictive of other clinical outcomes, namely multiple organ failure and mortality.

4.3 Methods

This was a single centre prospective cohort study of patients presenting to a Major Trauma Centre over a twenty four month period from August 2011.

4.3.1 Patient selection

Severely injured adult patients (>15 years), who met local criteria for full trauma team activation and were subsequently admitted to the critical care unit were enrolled. Patients who survived beyond 48 hours with an ISS>15, and had complete WBC, neutrophil, monocyte and lymphocyte counts from admission to day seven were included in the study.

4.3.2 Data collection

Data were collected prospectively on patient demographics, mechanism (blunt or penetrating injury), baseline physiology, admission immune cell profiles and transfusion

requirements in the first 24 hours from injury. Admission shock was defined as BD ≥6 mEq/L (208). Patients were reviewed daily in the critical care unit and followed up to discharge or in hospital death.

4.3.3 Immune cell analysis

WBC, neutrophil, monocyte and lymphocyte counts were collected from the day of admission until day seven of the inpatient stay. The need for blood draw was determined by the clinical team independent of the study. If patients had several blood tests within a 24 hour period, the most abnormal measurements were recorded. The procedures for WBC, neutrophil, monocyte and lymphocyte sampling and analysis are described in chapter 2.5.1.

4.3.4 Outcome measures

The primary outcome measure was the development of infection. Each participant was reviewed daily for the presence and timing of infection. The Centre for Disease Control and Prevention (CDC) criteria was used to define infection occurring ≥48 hours post admission (209). Infection was confirmed by direct observation of purulent exudate, or a combination of adverse clinical signs e.g. pyrexia or pulmonary infiltrates on a chest x-ray (not attributed to acute lung injury) and positive microscopy and culture. Further detail about the criteria for defining infection is described in chapter 2.4.1.

The main secondary outcomes were the development of multiple organ failure (MOF) and mortality. The development of MOF was assessed daily using the Sequential Organ Failure Assessment (SOFA) score (176). Single organ failure was defined as a SOFA score of ≥3 in one organ system during a 24 hour period. MOF was defined as single organ failure in two or more systems during a 24 hour period. Patients were followed until hospital discharge or death. For mortality analysis patients surviving to hospital discharge were assumed to be alive.

4.3.5 Data analysis

Statistical analysis was performed using SPSS v21 (IBM Corporation, Armonk, NY, USA). Students t tests or ANOVA were used to analyse parametric data and Mann Whitney U test or Kruskal Wallis test were used for non-parametric data analysis. The chi-square or Fisher's exact tests were used to analyse proportions and categorical variables. A p value of <0.05 was considered statistically significant.

A binary logistic regression model was created to identify factors including immune cell counts that were independently associated with infection. Initially, univariate statistical analysis was performed to examine the unadjusted effects of potential predictor variables. Those variables achieving a significance of $p<0.15$ were added to a multivariate logistic model for regression analysis.

4.4 Results

During the study period, 398 patients were admitted following trauma team activation. Fourteen (4%) patients died within the first 48 hours. 280 patients had immune cell blood tests taken from admission to day seven, therefore this comprised the final cohort. Overall, the cohort was predominantly male, severely injured (ISS 29) following blunt trauma, with a mean admission BD of 4.3 mEq/L [CI: 1.7-7.6] (Table 4. 1).

Table 4.1 Admission characteristics, immune cell counts and hypoperfusion

	All	No shock (BD<6 mEq/L)	Shock (BD≥6 mEq/L)
N	**280**	**176**	**104**
Male (%)	222 (79)	137 (78)	85 (82)
Age†	39 (26 – 54)	42 (27 – 57)	36 (26 – 51)
Blunt (%)	259 (93)	165 (94)	94 (90)
BD (mEq/L) †	4.3 (1.7 – 7.6)	2.3 (0.7 – 4.0)	9.1 (7 – 13.9)**
ISS†	29 (22 – 38)	26 (22 – 34)	34 (24–43)**
PRBC†	0 (0-1)	2 (0-6)	6 (3-11)**
WBCs^			
Day 1	15 (14.2 – 15.8)	14.9 (13.9 – 15.8)	15.3 (13.7 – 16.8)
Day 2	10.6 (10.1 – 11.1)	10.9 (10.2 – 11.6)	10 (9.2 – 10.9)
Day 3	10.2 (9.7 – 10.7)	10.6 (9.9 – 11.2)	9.5 (8.6 – 11.3)
Day 4	9.3 (8.8 – 9.8)	8.8 (8.1 – 9.4)	8.5 (7.7 – 9.3)
Day 5	9.6 (9.1 – 10.3)	9.9 (9.3 – 10.5)	9 (8.2 – 9.9)
Day 6	9.3 (9.8 – 10.8)	10.3 (9.7 – 10.9)	10.3 (9.3 – 11.2)
Day 7	12 (11.4 – 12.7)	11.7 (11 – 12.5)	12.5 (11.3 – 13.7)
Neutrophils^			
Day 1	12.1 (11.4 – 12.8)	11.8 (11.0 – 12.6)	12.6 (11.1 – 14.0)
Day 2	8.6 (8.1 – 9.0)	8.7 (8.1 – 9.3)	8.3 (7.6 – 9.1)
Day 3	8.2 (7.7 – 8.6)	8.4 (7.9 – 8.9)	7.8 (7.0 – 8.5)
Day 4	7.5 (7.0 – 8.0)	7.4 (7.3 – 8.6)	6.8 (6.1 – 7.5)
Day 5	7.4 (7.0 – 7.8)	7.1 (7.0 – 8.2)	6.8 (6.1 – 7.6)
Day 6	7.7 (7.3 – 8.2)	7.2 (7.1 – 8.3)	7.7 (6.9 – 8.6)
Day 7	8.8 (8.3 – 9.4)	8.7 (8.1 – 9.3)	9.0 (8.1 – 10.1)
Monocytes^			
Day 1	1.1 (1.0-1.2)	1.2 (1.0-1.4)	1.0 (0.9-1.2)
Day 2	0.9 (-0.8-1.0)	1.0 (0.8-1.1)	0.9 (0.8-1.1)
Day 3	0.7 (0.7-0.8)	0.8 (0.7-0.9)	0.7 (0.6-0.8)
Day 4	0.7 (0.6-0.7)	0.7 (0.6-0.8)	0.7 (0.6-0.7)
Day 5	0.8 (0.8-0.9)	0.8 (0.7-0.9)	0.8 (0.8-0.9)
Day 6	1.0 (0.9-1.1)	1.0 (0.9-1.2)	1.0 (0.9-1.1)
Day 7	1.2 (1.1-1.3)	1.2 (1.1-1.2)	1.0 (0.9-1.3)
Lymphocytes^			
Day 1	1.8 (1.6 – 2.1)	1.7 (1.5 – 1.9)	2.1 (1.6 – 2.6)
Day 2	1.0 (1.0 – 1.1)	1.0 (1.0 – 1.1)	1.0 (0.8 – 1.1)
Day 3	0.9 (0.9 – 1.0)	1.0 (0.9 – 1.1)	0.9 (0.8 – 1.0)
Day 4	0.9 (0.9 – 1.0)	1.0 (0.9 – 1.0)	0.8 (0.7 – 0.9)*
Day 5	1.0 (0.9 – 1.1)	1.1 (1.0 – 1.2)	0.8 (0.8 – 1.0)*
Day 6	1.2 (1.1 – 1.2)	1.2 (1.1 – 1.3)	1.1 (1.0 – 1.2)
Day 7	1.4 (1.3 – 1.5)	1.5 (1.3 – 1.7)	1.2 (1.1 – 1.4)
Outcomes			
Infection (%)	153 (55)	86 (48)	67 (64)*
Mortality (%)	24 (9)	14 (8)	10 (10)
MOF (%)	134 (47)	73 (41)	61 (59)*

Values expressed as median (IQR) †, Mean (95% CI) ^ or n (%). BD: Base Deficit; ISS: Injury Severity Score; PRBC: Packed red blood cells transfused in first 24 hours post injury; WBC: White Blood Cells; MOF: Multi-organ failure. Comparisons are between two cohorts: ** p<0.01; * p=0.01. Barts NHS Trust laboratory ranges: WBC 4-11 10^9/L; Neutrophils 2.5-7.5 10^9/L; Monocytes 0.2-0.8 10^9/L, Lymphocytes 1-4 10^9/L.

In all patients, the time course changes in immune cell counts after injury followed an expected initially pro-inflammatory response in the first 24-48 hours after admission (Table 4.1). White cells, neutrophils and monocytes were initially elevated and then returned to normal values during this period. Lymphocytes were also initially elevated, and then fell to lower than normal values, where patients remained lymphopenic until day four post injury. More than half of all of the study population (n=153, 55%) developed infection in the first seven days after injury, with median time to first infection at day four, principally affecting the respiratory tract.

4.4.1 Admission hypoperfusion and immune cell counts

On arrival to hospital, 37% of the cohort was in a state of shock (BD≥ 6 mEq/L). For both shocked and non-shocked patients, white cell counts were raised on admission, returning to normal limits until day seven (Table 4.1). Thereafter, mild leukocytosis was observed although there were no differences between groups.

Elevated neutrophils were observed in the first 72 hours for all cohorts. Neutrophil counts returned to normal until day seven when neutrophilia was observed, although this was not significant (Table 4.1). For all patients, non-significant rises in monocyte count were seen in the first two days from injury (Table 4.1). This was followed by a return to normal values on days three through five, with non-significant monocytosis observed on days six and seven.

Patients in shock were more lymphopenic between days three and five post injury (Table 4.1), with significant differences seen between groups on days four and five ($p=0.01$). Further analysis of the non-shocked and shocked cohorts indicated a persistent lymphopenia between days three and five in shocked patients, whereas non-shocked patients were never lymphopenic (Figure 4.1).

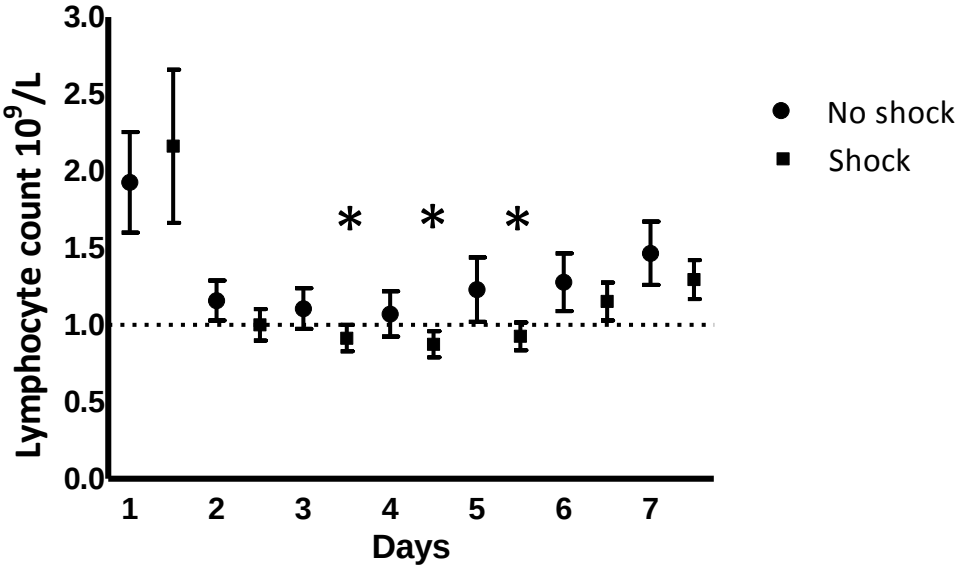


Figure 4.1. Daily lymphocyte counts and shock. Graph shows mean and 95 confidence intervals (error bars). Broken lines indicate normal ranges. Significant differences were seen between cohorts on days 3-5, with a trend to significance on day 2. Day 1: p=0.46, day 2: p=0.05, day 3: p=0.01, day 4: p=0.01, day 5: p<0.01, day 6: p=0.24, day 7: p=0.14.

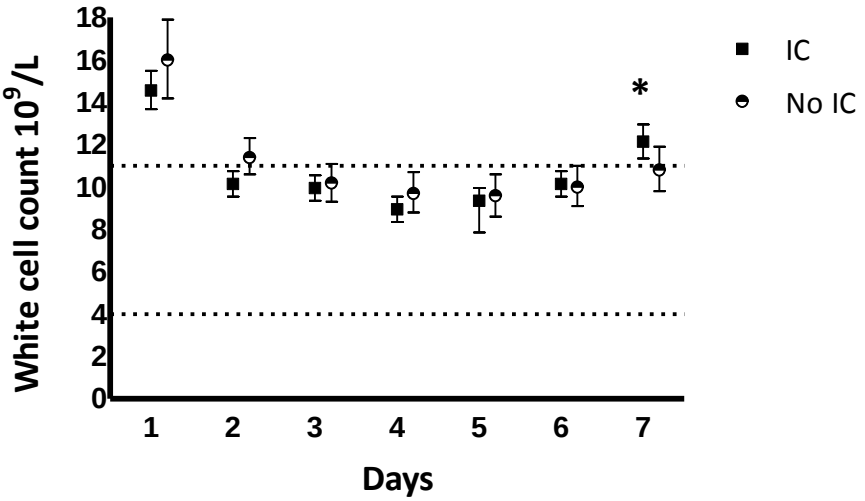
In summary, significant changes in early immune cell counts in the presence of shock were only observed in lymphocyte populations.

4.4.2 Immune cell profiles after severe injury

Overall white cell counts were above normal in all patients on admission to hospital, dropping to normal by day two. Only on day seven post injury was a significant difference between those patients with and without infection observed (Figure 4.2A). There were no differences in neutrophil counts between patient groups in the first seven days following admission. Neutrophilia was present in both groups between admission and 72 hours, and then dropped to normal levels until day six, when neutrophilia reoccurred (Figure 4.2B). Monocyte counts were raised on days one and two, and again at days six and seven, however significant differences were not seen between those who subsequently developed infection and those who did not (Figure 4.2C). However, lymphocyte counts differed between the two groups, with lymphopenia persisting from day two to six in patients who developed infection (Figure

4.2D). In the infection group, lymphocyte counts were significantly lower between days three and six, only returning to normal by day seven.

A



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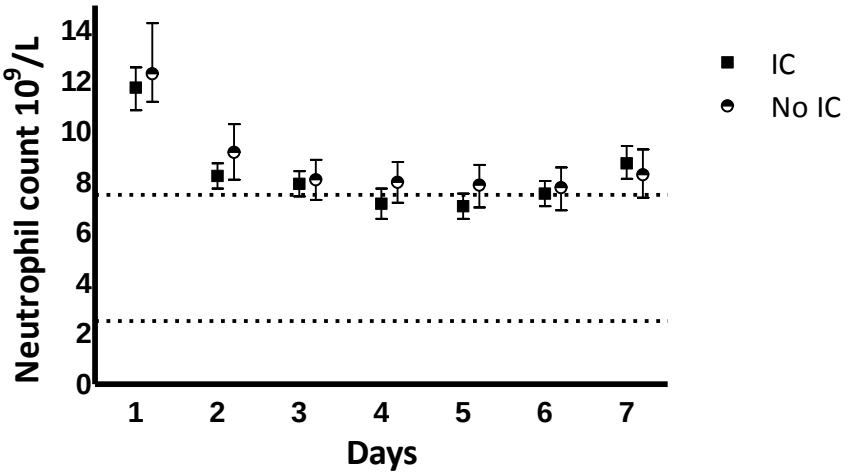

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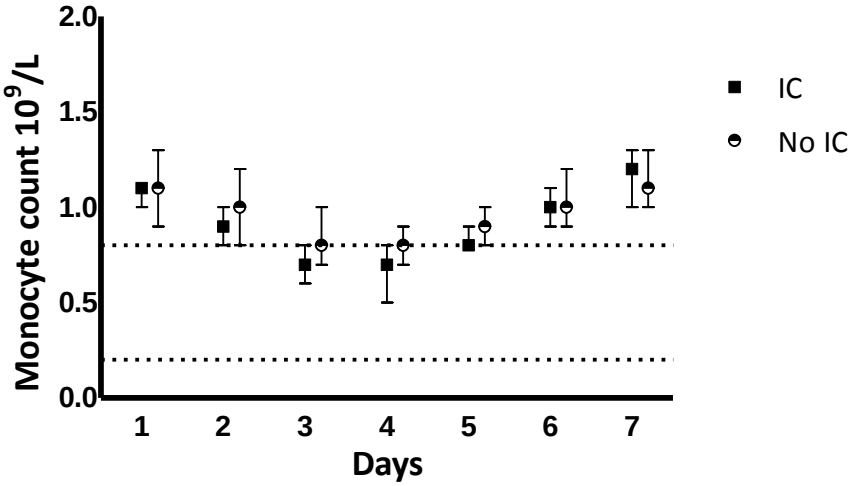

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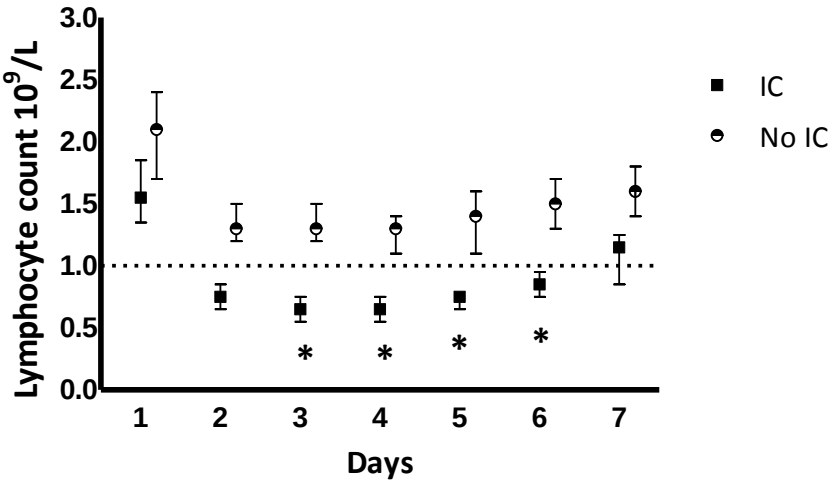


Figure 4.2 Early immune cell counts and infection. Graphs show mean and 95 confidence intervals (error bars). Broken lines indicate normal ranges. IC: Infectious complications.

A. **Early white blood cell counts and infection.** There were no differences in white cell counts between patients who developed infection and those who did not, until day 7 (No infection: 10.6 [9.6 – 11.7] vs. Infection: 12.4 [11.6 – 13.2], p<0.05). **B. Early neutrophil counts and infection.** There were no significant differences in neutrophil counts between patient groups in the first seven days following admission. **C. Early monocyte counts and infection.** There were no significant differences in monocyte counts between patient groups in the first seven days following admission. **D. Early lymphocyte counts and infection.** Patients who developed infection were lymphopenic from days 2-6. Day 3 (No infection: 1.1 [1 – 1.3] vs. Infection: 0.8 [0.8-1], p<0.001), day 4 (No infection: 1.1 [0.9 – 1.2] vs. Infection: 0.8 [0.8-1], p<0.001), day 5 (No infection: 1.2 [0.9 – 1.4] vs. Infection: 0.9 [0.9 - 1], p=0.01), and day 6 (No infection: 1.3 [1.1 – 1.5] vs. Infection: 1.1 [1 – 1.2], p=0.01).

Table 4.2 Multivariate logistic regression of variables independently associated with infection following severe injury

Univariate analysis		Multivariate analysis		
Variable	**p value**	**Odds ratio**	**95% CI**	**p value**
Age	0.51	x		
Gender	0.64	x		
Mechanism	0.72	x		
BD (mEq/L)	0.07*	1.14	1.04 – 1.23	0.02
ISS	0.47	X		
PRBC	0.56	X		
WBC (10^9/L)				
Day 1	0.31	X		
Day 2	0.22	X		
Day 3	0.67	X		
Day 4	0.62	X		
Day 5	0.66	X		
Day 6	0.38	X		
Day 7	0.02*	1.26	1.00 – 1.58	0.05
Neutrophils (10^9/L)				
Day 1	0.32	X		
Day 2	0.18	X		
Day 3	0.63	X		
Day 4	0.50	X		
Day 5	0.81	X		
Day 6	0.40	X		
Day 7	0.05*	0.90	0.70 – 1.16	0.45
Monocytes (10^9/L)				
Day 1	0.55	X		
Day 2	0.38	X		
Day 3	0.08*	0.94	0.24 – 3.59	0.93
Day 4	0.06*	1.40	0.37 - 5.25	0.61
Day 5	0.49	X		
Day 6	0.73	X		
Day 7	0.58	X		
Lymphocytes (10^9/L)				
Day 1	0.88	X		
Day 2	0.06*	X		
Day 3	0.01*	0.05	0.01 – 0.34	<0.01
Day 4	0.01*	0.10	0.02 – 0.48	<0.01
Day 5	0.03*	0.28	0.07 – 1.06	0.06
Day 6	0.06*	1.49	0.64 – 3.47	0.35
Day 7	0.88	X		

CI: Confidence interval; BD: Base Deficit; ISS: Injury Severity Score; PRBC: Packed red blood cells transfused in first 24 hours post injury; WBC: White Blood Cells. * univariate significant variable entered into the multivariate model.

Multivariate logistic regression was performed to identify a potentially predictive relationship between admission variables, immune cell counts and infection (Table 4.2). Three variables were independently associated with infection after trauma (R^2: 0.61). The presence of shock on admission had an independent relationship with infection, supporting the findings from chapter three. Low lymphocyte counts present on days three and four post injury were strongly associated with infection (Day 3: Odds ratio 0.05, 95% CI 0.01 – 0.34, p<0.01; Day 4: Odds ratio 0.10, 95% CI 0.02 – 0.48, p<0.01). These time points correlated with median time to infection. No relationship was found between leukocyte, neutrophil or monocyte counts and the development of infection in this cohort of patients.

4.4.3 Lymphopenia and the sites and timing of injury related infection.

The previous analysis demonstrated an independent relationship between lymphocyte counts at days three and four following injury and infection. The next aim was to evaluate if lymphopenia was predictive of the timing and site of first infection. In order to determine a predictive, diagnostic cut- off value for lymphopenia and the development of infection, a receiver operating characteristic (ROC) curve was constructed (Figure 4.3).

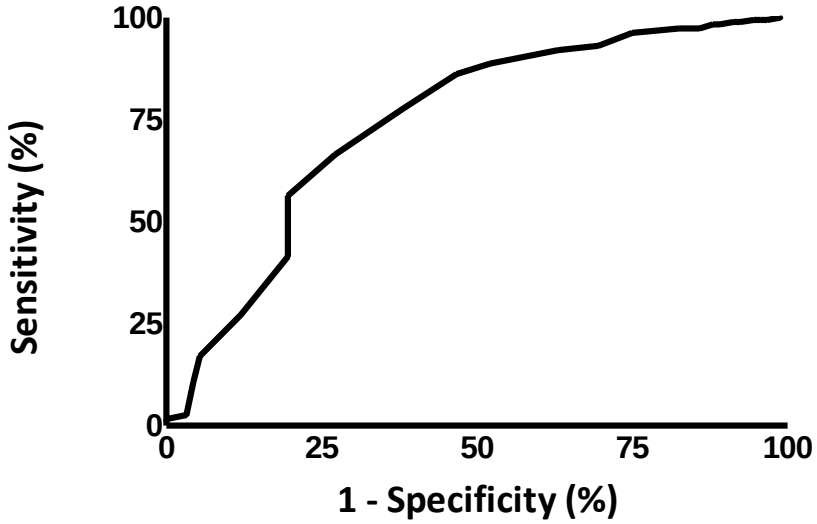


Figure 4.3. ROC curve: Lymphocyte counts predictive of the development of infection.

At a lymphocyte count of ≤0.8 10^9/L, the sensitivity was 67% and the specificity was 80% (Table 4.3), therefore this was chosen as the cut-off value for lymphopenia to be predictive of infection. The area under ROC (AUROC) was 0.74, which suggests that this is a fair test of diagnostic or predictive accuracy.

Table 4.3 Sensitivity and specificity of lymphocyte counts

Lymphocyte value	Sensitivity (%)	Specificity (%)
≤1.0 10^9/L	78	62
≤0.9 10^9/L	60	73
≤0.8 10^9/L	67	80
≤0.7 10^9/L	41	80
≤0.6 10^9/L	27	88
≤0.5 10^9/L	17	95

Infection rates were compared between those patients who were never lymphopenic (≥0.9 10^9/L) and those who had experienced at least one episode of lymphopenia. The majority of patients (70%) who developed infection had been lymphopenic (≤0.8 10^9/L) at least once after the initial 48 hours post injury time point.

The time to first infection significantly correlated with lymphocyte count changes (Figure 4.4). Median time to first infection from admission for patients in the lymphopenic cohort was day four, compared to day six for those never lymphopenic ($p<0.001$).

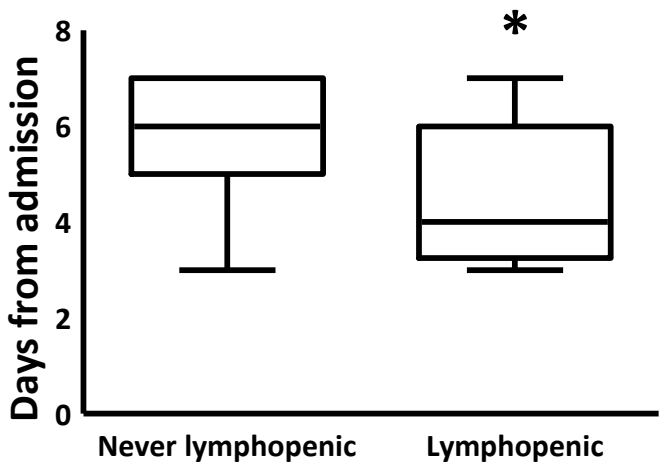


Figure 4.4 Median time to first infection. Box and whisker plots show median time to first infection for patients who were never lymphopenic compared to those who had at least one episode of lymphopenia. Never lymphopenic: 6 days (IQR 5-7) vs. Lymphopenic: 4 days (IQR 3-6), p<0.001.

Lymphopenia also correlated with diagnosis of new infection. There was a fifteen-fold increase in percentage of infections diagnosed on day three (Never lymphopenic: 6% vs. lymphopenic: 94%, p<0.001), and a six-fold increase on day four for those with lymphopenia (Never lymphopenic: 14% vs. lymphopenic: 86%, p<0.001) (Figure 4.5).

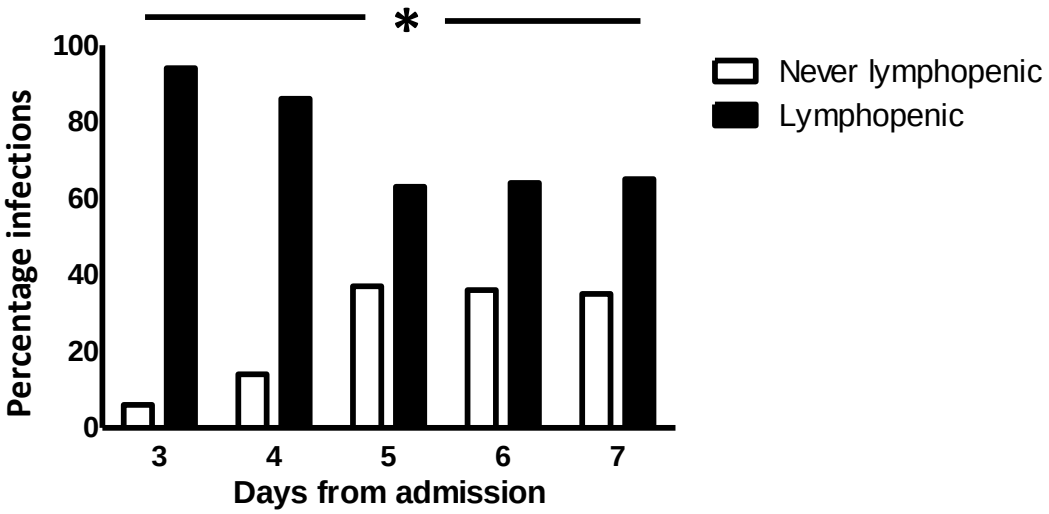


Figure 4.5 First day of infection and lymphocyte counts. Bar chart shows percentage of infectious episodes per first day of infection between never-lymphopenic and lymphopenic patients. Those who were lymphopenic at any stage had significantly greater percentage of infection from days 3-7, p<0.001.

The most common site of first infection for all patients was the respiratory tract. Lymphopenic patients had significantly higher rates of pneumonia on days three and four compared to those with normal counts (Day 3 - Normal count: 15% vs. Lymphopenia: 51% $p<0.01$, and Day 4 - Normal count: 22% vs. Lymphopenia: 44% $p<0.01$) (Table 4.4). There was a higher incidence of infection across all sites at these time points for lymphopenic patients, however numbers were small and this did not achieve statistical significance (Table 4.4).

Table 4.4 The effect of lymphopenia on timing and site of new infections

	Day 3		**Day 4**		**Day 5**		**Day 6**		**Day 7**	
L. count (10^9/L)	≤0.8	>0.8	≤0.8	>0.8	≤0.8	>0.8	≤0.8	>0.8	≤0.8	>0.8
RTI	20 (51)*	6 (15)	18 (44)*	9 (22)	12 (36)	10 (30)	11 (42)	12 (46)	4 (29)	5 (36)
BSI	3 (8)	0	4 (10)	3 (7)	1 (3)	2 (6)	0	0	1 (7)	1 (7)
UTI	0	0	0	0	1 (3)	1 (3)	1 (4)	0	0	1 (7)
TW	4 (10)	2 (5)	4 (10)	2 (5)	5 (15)	0	1 (4)	0	1 (7)	0
SSI	1 (3)	0	0	0	1 (3)	0	0	0	0	1 (7)
VAD	2 (5)	1 (3)	1 (2)	0	0	0	0	1 (4)	0	0
GI	0	0	0	0	0	0	0	0	0	0
Total daily new infection (n=153)	**39**		**41**		**33**		**26**		**14**	

Values are presented as n (%). L. count: Lymphocyte count; RTI: Respiratory tract; BSI: blood stream infection, UTI: urinary tract infection, TW: traumatic wound infection, SSI: surgical site infection, VAD: vascular access device infection, GI: gastrointestinal infection. *$p<0.001$

Overall, changes to lymphocyte counts at days three and four from admission were associated with the incidence, timing and site of infection. Lymphopenic patients suffered more infections at an earlier time point than patients with normal lymphocyte counts. The respiratory tract was the most prevalent site of infection, and lymphopenia was associated with significantly more episodes of respiratory infections early in the patients clinical course.

4.4.4 Lymphocyte counts and outcomes

Having established the strong association between lower lymphocyte counts and infection, the last aim was to evaluate a potentially predictive relationship between lymphopenia and other outcomes, namely multiple organ failure (MOF) and mortality.

4.4.4.i Lymphocyte counts and multiple organ failure

Overall, 134 (49%) patients developed MOF whilst in critical care. There were significant differences in daily lymphocyte counts between the MOF and non MOF cohorts. In patients who developed MOF there was a significantly lower lymphocytes on days three to six post injury (Table 4.5).

Table 4.5 Daily lymphocyte counts and MOF

	No MOF	MOF	p value
Lymphocytes 10^9/L			
Day 2	1.1 (1.0-1.2)	1.0 (0.9-1.1)	0.18
Day 3	1.0 (0.9-1.1)	0.9 (0.8-1.0)	0.01
Day 4	1.1 (1.0-1.2)	0.9 (0.8-0.9)	<0.01
Day 5	1.1 (1.0-1.2)	0.9 (0.8-1.0)	<0.01
Day 6	1.3 (1.2-1.4)	1.0 (1.0-1.1)	0.02
Day 7	1.5 (1.2-1.7)	1.4 (1.2-1.5)	0.24

Values are presented as mean (95% Confidence Intervals).

Therefore, the relative risk of developing MOF at these time points was calculated (Figure 4.6).

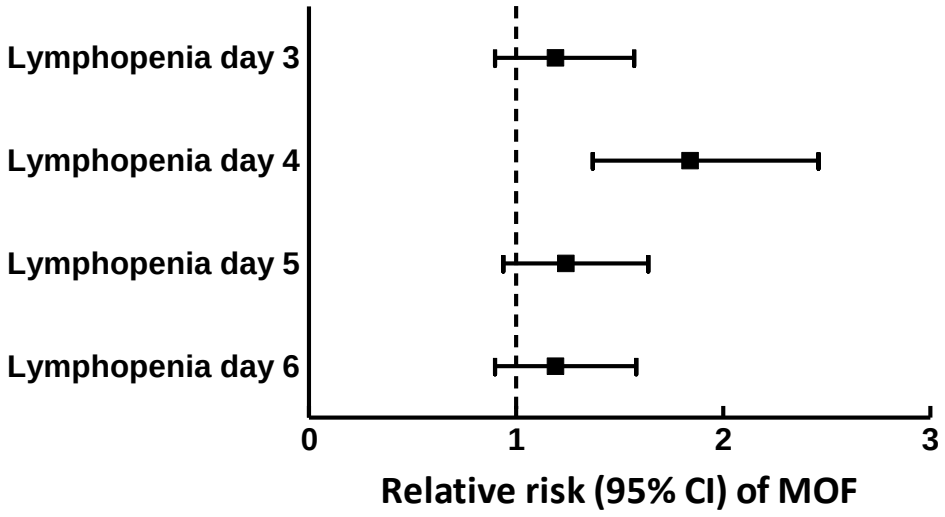


Figure 4.6 The relative risk of lymphopenia and the association with MOF. Relative risk of MOF (with 95% Confidence Intervals) is shown for each group. Broken line represents a relative risk of 1. Lymphopenia vs. normal lymphocyte count on day 3 p=0.20, lymphopenia vs. normal lymphocyte count on day 4 p<0.01, Lymphopenia vs. normal lymphocyte count on day 5 p=0.22, lymphopenia vs. normal lymphocyte count on day 6 p=0.21.

On days three, five and six, there was a trend to an increased risk of MOF associated with lymphopenia. However on day four the risk of developing MOF was significantly associated with the presence of lymphopenia (RR: 1.84 95% CI 1.37-2.46, p<0.01).

4.4.4.ii Lymphocyte counts and mortality

In the overall study cohort there was a 9% in-hospital (>48 hour) mortality rate. Changes in lymphocyte counts were analysed for patients who died compared with those who survived to discharge (Table 4.6).

Table 4.6 Daily lymphocyte counts and in-hospital mortality

	Alive to discharge	In-hospital mortality	p value
Lymphocytes 10^9/L			
Day 2	1.0 (1.0-1.1)	0.9 (0.8-1.1)	0.49
Day 3	1.0 (0.9-1.1)	0.7 (0.6-0.9)	0.01
Day 4	1.1 (1.0-1.1)	0.7 (0.6-0.9)	<0.01
Day 5	1.2 (1.0-1.1)	0.8 (0.7-0.9)	0.01
Day 6	1.2 (1.1-1.3)	1.0 (0.8-1.2)	0.10
Day 7	1.4 (1.3-1.6)	1.2 (0.9-1.4)	0.22

Values are presented as mean (95% Confidence Intervals).

Mean lymphocyte counts were lower every day for patients who died, with significant differences between groups on days three to five. Therefore, the relative risk of death at these time points was calculated, comparing lymphopenia with normal counts on days three to five (Figure 4.7).

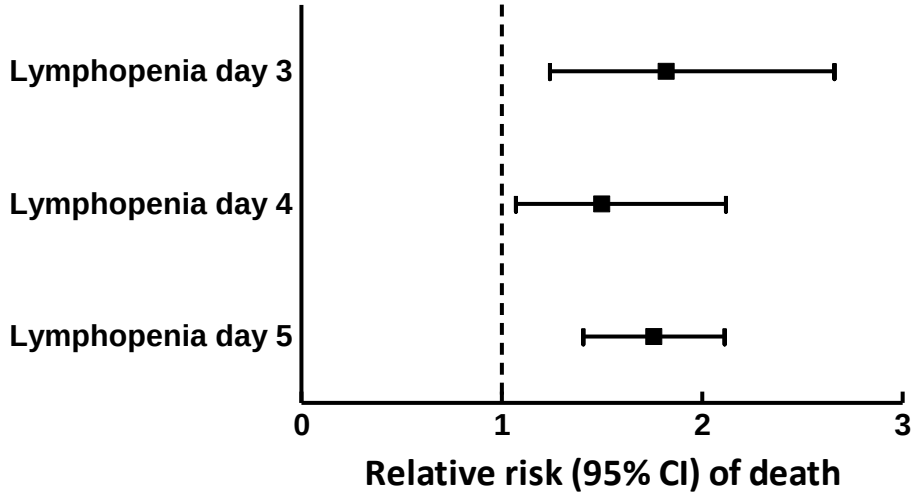


Figure 4.7 The relative risk of lymphopenia and the association with in-hospital mortality. Relative risk of death (with 95% Confidence Intervals) is shown for each group. Broken line represents a relative risk of 1. Lymphopenia vs. normal lymphocyte count on day 3 p=0.01, lymphopenia vs. normal lymphocyte count on day 4 p=0.03, lymphopenia vs. normal lymphocyte count on day 5 p=0.02.

Severely injured patients who were lymphopenic between days three and five following admission had a statisticially greater risk of death than patients with normal counts.

Lymphopenia which had not normalised by day five appeared to be predictive of in hospital mortality for severely injured patients.

4.5 Discussion

This study has demonstrated that changes to lymphocyte counts following traumatic injury were associated with the development of infection and other outcomes. Overall, there was a very high incidence of infection in this cohort of severely injured patients with a critical care stay of at least seven days. Decreased lymphocyte counts persisting to day five post injury correlated with the presence of hypoperfusion on admission. Lymphopenia, persisting to day four post injury, was associated with increased rates of infection, which developed earlier and resulted in more episodes of pneumonia. Lymphopenia present at day four was associated with the development of MOF, and if unresolved by day five post injury was predictive of in-hospital mortality. Low lymphocyte counts which do not return to normal levels after severe injury are strongly predictive of adverse outcomes.

There was a significant increase in infectious episodes for patients who were shocked on arrival, supporting the findings from the research described in chapter three. After the initial inflammatory response to injury, there were no differences in WBC, neutrophil or monocyte counts regardless of admission hypoperfusion. However lymphopenia persisted to post injury day five in those patients who presented in a shocked state. Lowered lymphocyte counts after traumatic injury have been reported previously (153, 162, 221), yet the associated presence of hypoperfusion has not been described in the evidence. Reasons for lymphopenia after trauma are not fully understood and possible suggested causes have included migration of lymphocytes into tissues in response to circulating catecholamines (221-223). This may explain the relationship between admission shock and lymphopenia observed in this study.

Lymphopenia on days three and four post injury was independently associated with infection in this severely injured cohort. Previous evidence suggests that levels of lymphocyte populations fall quickly following injury, with values generally returning to normal on day three (143, 221). In a non trauma critical care setting, 'severe' lymphopenia (defined as <0.8 10^9/L) still present at three days following admission was associated with a 53% sepsis rate (160). In trauma patients, tissue damage correlating with injury severity has been shown to result in depressed lymphocyte-driven cell mediated immunity (157). This may predispose severely injured patients to an increased risk of infections. The strong relationship revealed in this study between lymphopenia and infection at days three and four may therefore have clinical relevance in the prediction of infection.

Lymphopenic patients developed infections earlier than those who were never lymphopenic, which was much earlier than previously published (71, 108, 109). In common with many trauma studies, pnuemonia and respiratory infections were the greatest burden for this severely injured cohort (83, 84, 86, 106). However, the presence of lymphopenia resulted in a significantly greater percentage of respiratory infection early in their clinical course compared to those who had never been lymphopenic. Again, lymphopenia persisting past the initial inflammatory phase may help with the identification of patients at risk of early, clinically significant infections.

Lymphopenia was associated MOF, significantly at day four post injury. Prolonged lymphopenia may reflect the development of a persistent inflammatory catabolic syndrome, known to be associated with worse outcomes (159). The majority of patients who died whilst in hospital remained lymphopenic post injury, and time to death was significantly shorter in this cohort. Lymphopenia still present between days three and five post injury was predictive of mortality. This supports exisiting historic evidence which suggested that lymphopenia maximal at three days post injury resulted in increased mortality (153). More recently, failure to normalise lymphopenia within four days of injury was strongly associated with in-hospital death (162). My findings support

this evidence and add that lymphopenia persisting to day five post injury is predictive of in-hospital mortality in severely injured patients.

This study has demonstrated a strong relationship between lymphopenia and the development of trauma infections, but does have limitations. Firstly, cell counts were measured for seven days post injury, and sampling for a longer time period may have yielded further results in patients who developed infection at a later stage in their clinical course. Secondly, 280 patients is a relatively small cohort. However in order to evaluate immune cell changes in during the inflammatory response to severe injury, patients with ISS>15 requiring critical care intervention were most likely to have experienced this. A further limitation of the study is that whilst it demonstrated a strong association between lymphopenia and infection, the mechanistic link between the two was not investigated, and requires further characterisation.

4.6 Conclusion

This is the first study to have evaluated daily immune cell counts for a full week post injury and their relationship with infection. Lymphopenia which failed to normalise by day three or four after injury was strongly associated with the development of infection after trauma. Patients with persistently decreased lymphocyte counts also developed infections more quickly and suffered greater rates of pnuemonia. Prolonged lymphopenia after injury may have utility in identification of patients at risk of infection and other adverse outcomes. This may represent a potential clinical indicator for early intervention and reduction in infection.

CHAPTER FIVE COAGULATION SYSTEM CHANGES AND SUSCEPTIBILITY TO INFECTION IN TRAUMA PATIENTS

5.1 Introduction

Coagulation changes are common after severe injury (193), and major haemorrhage results in early acute traumatic coagulopathy (ATC) in approximately one quarter of patients (183). Coagulation and inflammation are closely linked, with common proteins and pathways allowing crosstalk between the two systems (190). In particular, the anticoagulant Protein C (PC) and fibrinolytic Plasminogen-Plasmin system, both implicated in the development of ATC, have strong immune regulatory properties (187, 224). Both proteins activate intracellular signalling pathways through their own cell surface receptors and activate immune cell mechanisms (21, 141, 225).

The importance of this crosstalk has been recognised in critically ill patients, where decreased protein C levels have been associated with the subsequent development of sepsis (226-229). Similarly, elevated plasmin levels have been associated with the development of gram negative bacterial infections in non-trauma critical care patients (230). It is possible therefore that change in these protein levels due to activation of anticoagulation and fibrinolytic pathways may lead to subsequent immunological deficits which leave trauma patients at risk of infection. The relationships between coagulation changes and the subsequent development of infection have not been established in trauma patients. Understanding these relationships may lead to opportunities for new therapeutic approaches in reducing the burden of infection.

5.2 Study objective and aims

The overall objective of this study was to define the relationship between the state of the coagulation system after injury and the subsequent development of infection.

First, to determine if the status of the coagulation system at 24 hours after injury was associated with the subsequent development of infection.

Secondly, to identify if there were any differences in the location of infection and infecting organisms associated with post-injury changes in the coagulation system.

Finally to examine clinical outcomes in patients who developed infection in the presence of coagulation changes.

5.3 Methods

5.3.1 Patient selection

All adult trauma patients (>15 years) who met the local criteria for full trauma team activation were eligible for enrolment into the ACIT II study (Activation of Coagulation and Inflammation in Trauma). Inclusion and exclusion criteria for ACIT II are described in detail in chapter 2.1.2. Patients who were subsequently admitted to critical care were enrolled into this study over a one year period.

5.3.2 Data Collection

Data were collected prospectively on patient demographics, mechanism of injury (blunt or penetrating force), baseline physiology and immune cell profiles. Arterial blood analysis for base deficit (BD) measurement was performed during the trauma team resuscitation on admission as part of normal processes of care. Blood product use in the first 24 hours following admission, namely Packed Red Blood cells (PRBC), Fresh Frozen

Plasma (FFP), Platelets and Cryoprecipitate, were documented. Injury severity was classified using the Injury Severity Score (29) once all injuries were accounted for.

5.3.3 Blood sampling technique

At 24 hours following admission a 20 mL research sample of blood was drawn along with the standard trauma laboratory tests (as per ACITII protocol). This time period was chosen to allow for completion of resuscitation and haemorrhage control processes. Samples for fibrinogen and coagulation factor assay were collected into 4.5ml glass vacutainers (0.109 M buffered sodium citrate, 3.2% - Becton Dickinson, Plymouth, UK). Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and D-Dimer (DD) were processed by the hospital laboratory along with a full blood count (FBC), neutrophils, monocytes and lymphocytes. Samples for Factor assay, PC and Protein S (PS) were centrifuged with double spun plasma extracted and stored at -80˚C within two hours of venepuncture. Blood for ROTEM analysis was drawn into a 2.7ml citrated vacutainer (0.109 M buffered sodium citrate, 3.2% - Becton Dickinson, Plymouth, UK) and processed in the trauma research laboratory.

5.3.4 Blood sample analysis

An automated analyser (Sysmex CA-1500 System, Siemens AG, Germany) was used to measure coagulation factors, PC and PS. Plasma activity levels of α2-antiplasmin were assayed using a Sysmex CS2100i automated analyser (Siemens AG, Germany). Enzyme linked immunosorbant assays were used to quantify tissue plasminogen activator (tPA; Asserachrom tPA, Diagnostica Stago, France) and plasmin-α2-antiplasmin complex (PAP; DRG PAP micro, Germany). Thromboelastometry was performed within one hour of blood draw at 37°C on a ROTEM delta instrument (TEM Innovations GmbH, Munich, Germany Clotting Time (CT), Clot Amplitude at 5 minutes (CA5), alpha angle (α angle) and maximum clot firmness (MCF) were reported for each sample analysed. More detailed description of sample analysis is provided in Chapter 2.6.

5.3.5 Outcome measures

The primary outcome was infection. This was defined using the Centre for Disease Control and Prevention (CDC) criteria as a 'localised or systematic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) occurring ≥48 hours post admission' (209). Infection was confirmed by direct observation of purulent exudate, adverse clinical signs e.g. pyrexia or pulmonary infiltrates on a chest x-ray and positive microscopy and culture. More detailed methods for the diagnosis of infection are described in chapter 2.4.1.

Secondary outcomes measured were in-hospital mortality, the development of organ failure (measured using the Sequential Organ Failure Assessment (SOFA) score), critical care length of stay (LOS) and total hospital LOS. Single organ failure (SOF) was defined as a SOFA score of ≥3 in one organ system during 24 hour period. Multiple organ failure (MOF) was defined as SOF in two or more organ systems during a 24 hour period (176). Organ failure scoring systems are described in more detail in chapter 2.4.2. Patients were followed until hospital discharge or death. For mortality analysis patients surviving to hospital discharge were assumed to be alive.

5.3.6 Data analysis

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego CA USA) and SPSS v10.0.5 (IBM Corporation, Armonk, NY, USA) for multivariate analysis. Non-parametric data were expressed as median (interquartile range). Parametric data were expressed as mean (95% confidence intervals). Mann Whitney U test or Kruskal Wallis test were used to analyse non-parametric data, and Students t test or ANOVA for parametric data. Percentages were analysed using Chi square or Fisher's exact tests. A p value of <0.05 was considered statistically significant.

5.4 Results

One hundred and fifty eight patients were recruited to the study over a twelve month period in accordance with the inclusion criteria. Patients who developed infection were older, more likely to have sustained blunt trauma and were more severely injured than those who did not develop infection. The presence of hypoperfusion on admission was significantly associated with infection (Table 5.1).

Table 5.1 Admission characteristics

	All (n=158)	**No infection (n=87)**	**Infection (n=71)**
Male	134 (85%)	73 (55%)	61 (45%)
Age^	35 (24-54)	32 (24-42)	44 (25-59)*
Blunt injury	133 (84%)	67 (77%)	66 (93%)
ISS^	15 (9-29)	15 (9-18)	25 (15-33)**
BD mEq/L†	2.5 (1.8–3.1)	1.1 (0.3–1.8)	4.2 (3.3-5.2)**
SBP <90 mmHg	25 (16%)	13 (15%)	12 (17%)
PRBC in 1st 24H†	1 (0.61.1)	1 (0.5-1.8)	2 (1.2-2.7)
FFP in 1st 24H†	0.5 (0.3-0.7)	1 (0.2-1.4)	1 (0.6-1.6)
Cryo in 1st 24H†	0 (0-0.1)	0 (0-0.2)	0 (0-0.3)
Plts in 1st 24H†	0 (0-0.1)	0 (0-0.3)	0 (0-0.3)
WBC 10^9/L†	9.7 (9.0-10.1)	9.6 (8.8-10.3)	9.6 (8.7-10.4)
Neutrophils 10^9/L†	7.5 (7.0-7.9)	7.2 (6.5-7.9)	7.7 (7.0-8.5)
Monocytes 10^9/L†	1.1 (1.0-1.2)	1.2 (1.0-1.4)	1.0 (0.9-1.1)
Lymphocytes 10^9/L†	1.2 (1.1-1.3)	1.3 (1.2-1.5)	1.0 (0.8-1.1)*

Values are ^median (inter quartile range), †mean (95% Confidence Intervals) or number (%).ISS: Injury Severity Score; BD: Base Deficit; SBP: Systolic Blood Pressure; PRBC: Packed Red Blood Cells; FFP: Fresh Frozen Plasma; Cryo: Cryoprecipitate; Plts: Platelets; WBC: White Blood Cells. Barts health laboratory ranges: WBC 4-11 10^9/L; Neutrophils 2.5-7.5 10^9/L; Monocytes 0.2-0.8 109/L; Lymphocytes 1-4 10^9/L. p value: comparison of two groups. * $p<0.05$; ** $p<0.01$

Immune cell counts at 24 hours after injury were analysed for those who subsequently developed infection compared to those who did not. WBC, neutrophil and monocyte counts were similar and within normal ranges for both groups of patients. However, there was a small but statistically significant difference in lymphocytes between groups (Table 5.1). Patients who subsequently developed infection had lower lymphocyte counts at 24 hours post injury (No infection: 1.3 [1.2-1.5] vs. Infection: 1.0 [0.8-1.1], p=0.03). Overall, there did not appear to be an obvious exhaustion of immune cells at this time-point after injury which might explain a subsequent susceptibility to infection. However, the trend to lymphopenia in those who developed infection supports the findings from the cohort study described in chapter four.

Analysis of the global status of coagulation was initially carried out using functional thromboelastometry testing (ROTEM). At 24 hours, all ROTEM parameters were similar in both groups with no statistical differences noted (No Infection vs. Infection – CT: 67sec vs. 62sec, CA5: 45mm vs. 44mm, alpha angle: 70.7mm vs. 70.5mm, MCF: 61mm vs. 60mm, all not significant). Laboratory tests of coagulation at 24 hours post injury (PT, APTT) differed between the two groups but remained within normal ranges (No infection vs. Infection - PT: 11.5sec vs. 12sec, p=0.06; APTT: 28.6sec vs. 32.7sec, p=0.04). Therefore, there were no obvious differences in functional clot formation at 24 hours between patients who subsequently developed an infection and those who did not.

Despite these normal functional clotting profiles there were significant underlying changes in the levels of the coagulation system constituents. Procoagulant factor levels were significantly lower in the infection group, although average values remained within the normal ranges (Table 5.2). Excluding factor VII, where levels were higher but not statistically significant, patients who developed infection had 24-hour procoagulant protein levels around 12% lower than those who did not (varied between factor VIII 6.4% and factor II 16.2%).

Table 5.2 Coagulation factors and infection

Test/Factor	Normal range	No infection	Infection	
Fib	1.50-4.50 g/l	2.96 (2.82-3.10)	2.67 (2.47-2.88)	**
II	78-117 iu/dL	76.4(72.1-80.7)	64 (59.3-68.6)	
V	66-114 iu/dL	87 (80.6-93.4)	75.2 (69.1-81.30)	
VII	50-150 iu/dL	62.1 (56.8-67.4)	62.9 (55-70.8)	
VIII	52-153 iu/dL	155.8 (145-166.6)	145.8 (132.2-156.4)	**
IX	58-138 iu/dL	108.2 (103.1-113.2)	99.2 (93.7-104.8)	**
X	50-150 iu/dL	82.2 (77.7-86.7)	70.8 (66.3-75.3)	*
XI	58-148 iu/dL	75 (70.8-79.2)	63.4 (59.4-67.4)	**
XIII	70-140 iu/dL	89.2 (84.2-94.1)	80.2 (74.2-86.1)	*
AT	81-119 iu/dL	89.5 (85.5-93.6)	78.8 (74.5-83.1)	*
PC	72-162 iu/dL	83.3 (78.5-88.1)	70.2 (65.4-74.9)	**
PS	62-120 iu/dL	86.4 (81-91.8)	75.8 (70-81.6)	
PAP	120-700 µg/L	3324 (2412-4236)	6156 (4397-7916)	**
a2ap	76-126 iu/dL	95.6 (90-100.9)	84.4 (79.5-89.2)	*
PAI-1	4-43 ng/mL	33.2 (24.3-42.2)	32.7 (23.9-41.5)	
tPA	2–12 ng/mL	11.3 (9.2-13.5)	14.4 (9.4-19.5)	
DD	<550 ng/mL	5422 (4242-6602)	9383 (7169-11600)	**

Values are expressed as mean (95% Confidence intervals). Fib: fibrinogen, AT: Anti thrombin III, PC: Protein C, PS: Protein S, PAP: Plasmin antiplasmin, a2ap: α_2-antiplasmin, PAI-1: Plasminogen activator inhibitor 1, tPA: tissue plasminogen activator, DD: D-dimer. *p<0.05; ** p=0.01

At 24 hours post injury, patients who went on to develop infection had depleted levels of anticoagulants Antithrombin III (11.9% reduction compared to no infection, p=0.04) and Protein S (12.2% reduction compared to no infection, p=0.09) (Table 5.2). Protein C

(PC) was significantly lower in the infection group (No infection: 83.3 iu/dL, vs. Infection: 70.2 iu/dL, p=0.01), (Table 5.2). Further analysis revealed a dose dependent increase in the development of infection as levels of PC decreased (Figure 5.1).

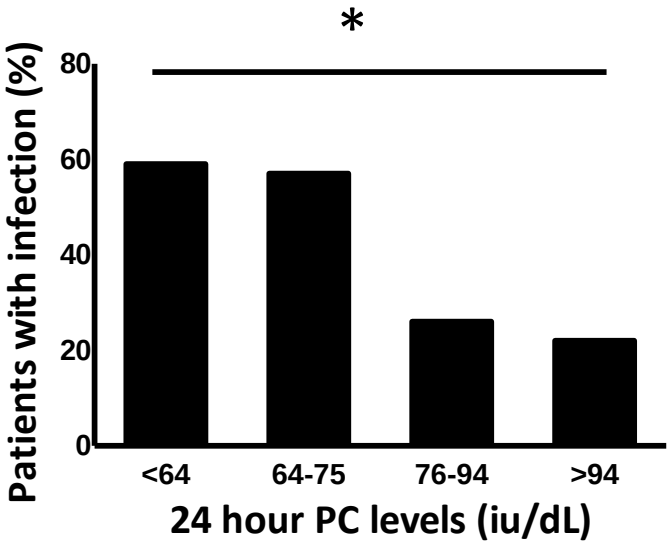


Figure 5.1 Percentage of patients with infection in 24 hours PC quartiles. Graph shows percentage of patients with infection in 24 hour PC quartiles (iu/dL).In <64iu/dL: 59% of patients developed infection, 64-75iu/dL: 57%, 76-94iu/dL: 26% and >94iu/dL: 22%; p<0.001 (chi squared test for trend).

For fibrinolytic pathways at 24 hours, levels of Alpha 2 antiplasmin (a2ap) and Plasminogen activator inhibitor 1 (PAI-1) remained within normal ranges for both groups of patients (Table 5.3). D-Dimer levels were significantly raised (No infection: 5422 ng/mL, vs. Infection: 9383 ng/mL, p=0.01), and tissue plasminogen activator (tPA) levels were 27.4% higher in the infection group although this was not statistically significant (Table 5.3). However, plasmin antiplasmin (PAP) levels were almost 50% higher in patients who developed infection (No infection: 3324 µg/L, vs. Infection: 6156 µg/L, p=0.01). Furthermore, the proportion of patients who went on to develop infection increased with plasmin generation (Figure 5.2).

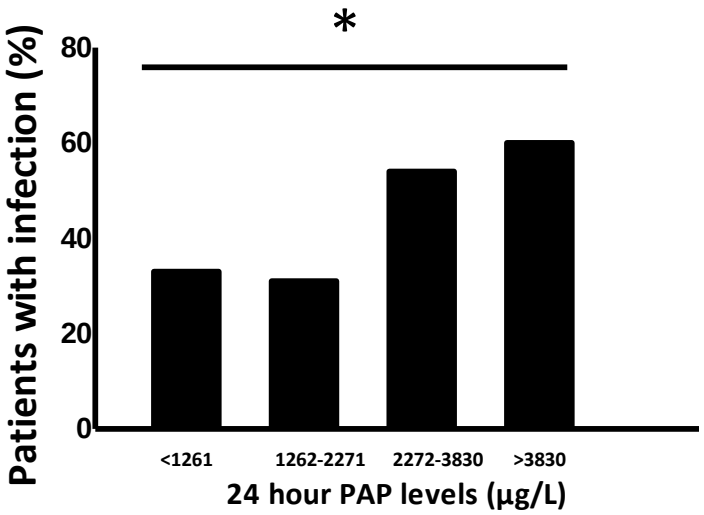


Figure 5.2 Percentage of patients with infection in 24 hour PAP quartiles. Graph shows percentage of patients with infection in 24 hour PAP quartiles (µg/L). In <1261µg/L: 33% of patients developed infection, 1262-2271µg/L: 31%, 2272-3830µg/L: 54% and >3830µg/L: 60%; p<0.001 (chi squared test for trend).

Most infections occurred in patients who had significantly raised levels of PAP complex with 65% of total infections observed in the two highest PAP quartiles (≥2272 µg/L).

Multivariate logistic regression analysis was performed to identify the coagulation factors that were independently associated with infection. The inter-connectivity of the coagulation and fibrinolytic system could have potentially complicated the analysis. Therefore factors found to be significant in univariate analysis were selected as representatives of their primary pathway and included in the model (II - procoagulant; PC – anticoagulant; PAP – fibrinolysis; Fibrinogen - substrate). In multivariate analysis only PC (Odds ratio 0.97, 95% CI: 0.97-0.99, p=0.004) and PAP (Odds ratio 1.01, 95% CI: 1.01-1.03, p=0.006) were independently associated with infection (R Squared=0.58). Thus at 24-hours after injury, patients who subsequently develop infections had independent differences in levels of coagulation components known to have important roles in immunological activity.

The majority of infections, both aerobic and anaerobic, occurred in patients who had significantly depleted levels of PC (≤75 iu/dL) at 24 hours (76% anaerobic bacteria and 74% aerobic bacteria), with most infections found in sputum, traumatic wounds and blood cultures (Table 5.3). This trend continued with gram positive and gram negative infections, where higher numbers of both were seen in the two lowest PC quartiles (Table 5.3).

Table 5.3 24 hour Protein C levels and outcomes

	24H PC <64	**24H PC 64-75**	**24H PC 76-94**	**24H PC >94**
N=158	42	42	38	36
Patients with infection: n = 71	25 (59%)	24 (57%)	14 (36%)	8 (22%)
Source of infection				
Sputum (n= 65)	27 (42%)	22 (34%)	11 (16%)	5 (8%)*
Traumatic wound (n = 51)	19 (37%)	19 (37%)	9 (17%)	4 (9%)*
Urine (n = 14)	9 (64%)	3 (22%)	1 (7%)	1 (7%)*
IV line (n=8)	4 (50%)	3 (37%)	1 (13%)	0 (0%)*
Blood culture (n=20)	13 (65%)	6 (30%)	0 (0%)	1 (5%)*
Microbes				
Gram positive (n=71)	27 (38%)	22 (31%)	10 (14%)	12 (17%)*
Gram negative (n=94)	25 (27%)	49 (52%)	18 (19%)	2 (2%)*
Aerobic (n = 39)	15 (38%)	14 (36%)	8 (21%)	2 (5%)*
Anaerobic (n = 109)	37 (34%)	46 (42%)	12 (11%)	14 (13%)*
Fungal (n = 6)	3 (50%)	2 (33%)	1 (17%)	0 (0%)*
Outcomes				
Mortality	3 (7%)	1 (2%)	2 (5%)	3 (8%)
Multiple organ failure	17 (40%)	13 (31%)	5 (13%)	7 (19%)*
Critical care LOS (days)	7 (4-12)	10 (5-13)	5 (2-8)	6 (2-11)
Hospital LOS (days)	13 (6-26)	13 (6-24)	11 (4-19)	6 (3-16)*

Values are shown as median (inter quartile range) or number (%). LOS: Length of Stay. * p <0.01, chi squared test for trend.

Similarly, more anaerobic and aerobic infections developed where plasmin levels had increased (PAP≥2272 μg/L: 70% anaerobic infection and 72% aerobic infection) (Table

5.4). Greater numbers of both gram positive and gram negative infections were seen in the highest PAP quartiles (Table 5.4).

Table 5.4 24 hour Plasmin-Antiplasmin levels and outcomes

	24H PAP <1261	24H PAP 1262-2271	24H PAP 2272-3830	24H PAP >3831
N=158	40	39	39	40
Patients with infection: n = 71	13 (33%)	12 (31%)	21 (54%)	25 (63%)
Source of infection				
Sputum (n = 65)	9 (14%)	5 (8%)	17 (26%)	34 (52%)*
Traumatic wound (n = 51)	9 (18%)	5 (10%)	22 (43%)	15 (29%)*
Urine (n = 14)	3 (22%)	2 (14%)	2 (14%)	7 (50%)*
IV line (n = 8)	1 (13%)	0 (0%)	4 (50%)	3 (37%)*
Blood culture (n = 20)	3 (15%)	2 (10%)	6 (30%)	9 (45%)*
Microbes				
Gram positive (n=71)	11 (15%)	9 (13%)	26 (37%)	25 (35%)*
Gram negative (n=94)	16 (17%)	9 (10%)	24 (25%)	45 (48%)*
Aerobic (n = 39)	9 (23%)	2 (5%)	14 (36%)	14 (36%)*
Anaerobic (n = 109)	17 (16%)	16 (14%)	24 (22%)	52 (48%)*
Fungal (n = 6)	0 (0%)	0 (0%)	3 (50%)	3 (50%)
Outcomes				
Mortality	3 (8%)	0 (0%)	1 (3%)	5 (13%)
Multiple organ failure	11 (28%)	8 (21%)	12 (31%)	12 (30%)
Critical care LOS (days)	5 (3-15)	4 (2-8)	7 (3-9)	8 (2-14)
Hospital LOS (days)	8 (3-17)	6 (4-16)	15 (7-23)	19 (7-30)*

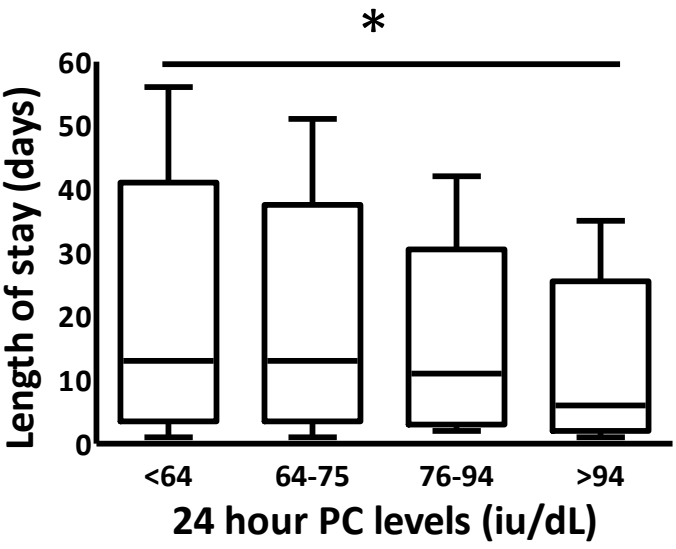
Values are shown as median (inter quartile range) or number (%). LOS: Length of Stay. * p <0.01, chi squared test for trend.

Overall mortality rates were low, (n=9, 6%). Mortality was higher with increased plasmin generation at 24 hours (Table 5.4) however this increase did not correlate with lower levels of PC. The incidence of multiple organ failure increased with depleted levels

of PC (Table 5.3, p<0.01), and although not statistically significant, rates of MOF also increased with raised levels of PAP (Table 5.4).

Critical care length of stay was greater in the lower PC quartiles (PC ≤75 iu/dL: 17 days vs. PC >75 iu/dL: 11 days, ns) and also with plasmin generation (PAP <2271 µg/L: 9 days vs. PAP ≥2272 µg/L: 15 days, ns). Significant differences were seen in total hospital length of stay in depleted PC quartiles (PC ≤75 iu/dL: 26 days vs. PC >75 iu/dL: 17 days, p=0.04) (Figure 5.3A) and for increased PAP levels (PAP <2271 µg/L: 14 days vs. PAP ≥2272 µg/L: 34 days, p=0.02) (Figure 5.3B).

A



B

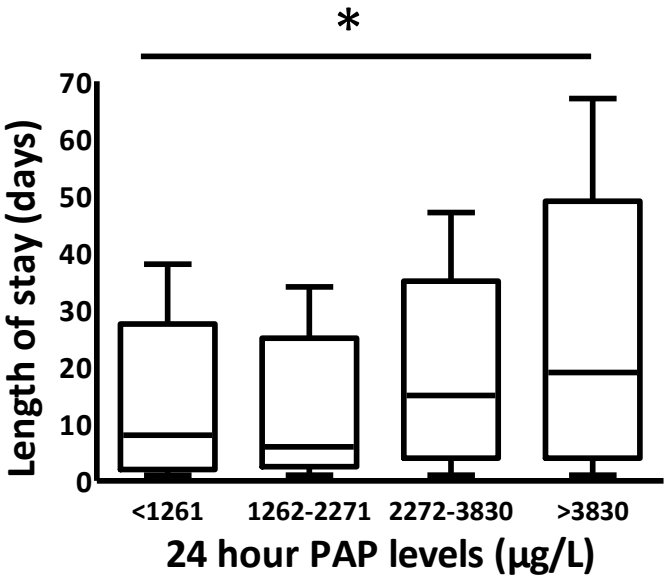


Figure 5.3. Coagulation changes and length of stay. A. Total length of hospital stay in 24 hour PC quartiles. Box and whisker plots show median, interquartile range and adjusted range of hospital length of stay of patients with infection in 24 hour PC quartiles (iu/dL). <64iu/dL: 13 days, 64-75iu/dL: 13 days, 76-94iu/dL: 11 days and >94iu/dL: 6 days; p=0.04 (Kruskal Wallis test). **B. Total length of hospital stay in 24 hour PAP quartiles.** Box and whisker plots show median interquartile range and adjusted range of hospital length of stay of patients with infection in 24 hour PAP quartiles (µg/L). <1261µg/L: 8 days, 1262-2271µg/L: 6 days, 2272-3830µg/L: 15 days and >3830µg/L: 19 days; p=0.02 (Kruskal Wallis test).

5.5 Discussion

This prospective study has examined the association between coagulation factor changes and infection in a trauma setting. Patients who subsequently developed infections had normal white cell and functional coagulation profiles at 24 hours, but showed depletion of PC and increased levels of PAP. These findings characterise a patient group at increased risk of infection and may also represent an opportunity for therapeutic intervention to improve outcomes after trauma.

Coagulopathy following severe injury is not uncommon and has been shown to have an adverse effect on morbidity and mortality (96, 231). ATC characterised by systemic anticoagulation with depleted levels of PC, and global fibrinolysis presents early in the patient course following injury (21, 63, 183, 188). Early activation of PC may lead to a subsequent depletion, and these patients are known to develop a late hypercoagulable

state and thrombotic risk (232, 233). PC depletion and sepsis is well described in studies examining outcomes following critical illness (227, 228, 234). In trauma patients, depletion of PC up to the first 12 hours after injury was associated with a propensity to nosocomial lung infection (217). The findings of this study add to the evidence and demonstrate that patients who do not recover their plasma PC levels at 24 hours post injury are significantly more likely to develop HAI, with lower PC levels correlating with higher rates of both gram positive and gram negative infections.

The coagulation system was traditionally viewed as entirely separate from the immune response. However, it is now suggested that coagulation and innate immunity have co-evolved and that following tissue injury their cross talk results in simultaneous activation (190). Non-trauma evidence has described the important role that the coagulation system plays in the host response to infection. D-dimers and PAP were found to be raised early in non-trauma sepsis, with a further increase in levels once severe sepsis was confirmed (235). These changes were independent of causative infectious pathogens with both gram positive and gram negative organisms associated with increased fibrinolysis. Furthermore, in non-trauma bacterial infections, PAI-1 and a2ap, both activators of plasmin, were reported to increase dispersal of fibrin clots thus facilitating bacterial spread (236). In trauma patients, this study adds to the evidence by revealing a strong association between activation of the plasminogen-plasmin system at 24 hours post injury and an increased risk of infection.

The development of infectious complications was associated with increased total hospital stays. Hospital length of stay was also strongly associated with PC depletion and increased plasmin-antiplasmin levels at 24 hours. Many factors affect hospital stay, however if these coagulation changes are causative, this may represent an opportunity for clinical intervention to reduce both the incidence of infection and hospital stays, and improve the patient experience.

This study has a number of limitations. Whilst I have demonstrated an association between coagulation changes and infection, the mechanistic, causal link between the two was not established, and requires further characterisation. Secondly, the selection of variables for regression analysis could have potentially impacted on the results. The independent variables within each pathway may have been highly correlated to one another therefore significant representatives of each were chosen. Forwards (addition) and backwards (elimination) stepwise regression technique might have been considered, which is useful for selecting and standardising a small set of the most significant predictor variables (237). Alternatively, variables may have been selected and reduced using Lasso regression (238), however this would have required advanced statistical expertise. Finally, this study did not specifically examine the acute phase of care to establish which interventions may have contributed to low PC and high PAP levels at 24 hours. However severity of injury was much higher in those who developed infection, as was the degree of admission hypoperfusion. This may reflect the level of tissue damage and haemorrhage present in these patients, which could explain the coagulation differences experienced by the infection cohort. Further research examining early interventions for trauma patients known to be or suspected of bleeding and the effect on infection is warranted.

5.6 Conclusion

Infection following severe injury develops in patients who have an abnormal coagulation profile at 24 hours, specifically those with in anticoagulant and fibrinolytic pathways. Decreased levels of PC and increased plasmin generation and other fibrinolytic markers at 24 hours post injury were predictive of infection. Early management of haemorrhage and potential coagulopathy may improve outcomes for this population, and this may represents an opportunity for further research.

CHAPTER SIX THE EFFECT OF TXA ON THE DEVELOPMENT OF INFECTION IN SEVERELY INJURED PATIENTS

6.1 Introduction

Hemorrhage following traumatic injury is a leading cause of global mortality and morbidity (193). Tranexamic acid (TXA) has demonstrated survival benefits in trauma patients in a single large multicentre randomized control trial (64) and a subsequent military study (203). This has led many services to include TXA in their major hemorrhage protocols (196, 204). Mortality benefits following early TXA use appear greatest in those patients who are the most shocked, requiring massive transfusion (191, 203). There is an on-going debate however as to whether TXA may be of benefit to all trauma patients, based on a prespecified analysis of the CRASH-2 results (239). Furthermore, while TXA may improve survival it may also be beneficial for other outcomes such as infection, given its known mechanisms of action (240). The interconnection between haemorrhage and inflammation is well described (187, 241), and TXA has anti-inflammatory properties (242). TXA blocks the plasminogen and plasmin receptor binding sites resulting in a decreased inflammatory response (206). Evidence from cardiac surgery suggests that pre-operative TXA may also attenuate inflammation through inhibition of fibrinolysis (243, 244).

The effects of TXA on infection and other non-mortality outcomes such as organ failure have not been described. In chapter three I described the relationship between admission shock and infection. In chapter five the presence of fibrinolysis at 24 hours post injury was found to be strongly associated with the development of infection. Consequently, the utility of TXA in the timely management of haemorrhage and coagulopathy may reduce the incidence of infection.

6.2 Study objective and aims

The overall objective of this study was to characterise the relationship between TXA use and clinical outcomes in a severely injured civilian cohort.

The primary aim was to assess the effect of TXA on the development of infection, and to determine any differential effect between patients who presented to hospital with and without shock.

Secondly, to evaluate the effect of TXA on multiple organ failure, mortality and other clinical outcomes.

6.3 Methods

6.3.1 Patient selection

All adult trauma patients (>15 years), admitted to the critical care unit following trauma team activation over a two year period were recruited. In order to focus on a severely injured cohort, all patients admitted to critical care were initially included and then those whose ISS was calculated to be <16 were retrospectively excluded. Overall injury severity was classified using the Injury Severity Score (ISS) (29).

The Emergency Department (ED) has a 'Code Red' major hemorrhage protocol (MHP) to guide blood product replacement during trauma resuscitation. At the start of the study period, TXA 1g was administered in the ED within three hours of injury, followed by a 1g infusion at the discretion of the trauma team leader when hemorrhage was detected or suspected. Three months into the study, TXA was formally introduced into the MHP, following the standard CRASH 2 protocol (64). Patients are given 1g in the first three hours following injury followed by a 1g infusion over eight hours. This initial dose is administered either by clinicians in pre hospital care (PHC) or the ED if the systolic blood

pressure (SBP) is <90mmHg, there is a poor response to an initial fluid bolus and there is suspected active hemorrhage.

6.3.2 Data collection

Data were collected prospectively on patient demographics, mechanism of injury (blunt or penetrating force), baseline physiology and clotting profiles. Arterial blood analysis for base deficit (BD) measurement was performed during the trauma team resuscitation on admission as part of normal processes of care. The presence of clinically significant shock known to be strongly associated adverse outcome was defined as a BD ≥6mEq/L (208). Time to operation or interventional radiology from admission was recorded. Crystalloid, PRBC, FFP, Platelets and Cryoprecipitate use in the first 24 hours following admission were documented.

6.3.3 Outcome measures

The primary outcome was infection, defined using the Centre for Disease Control and Prevention (CDC) criteria as a 'localised or systematic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) occurring ≥48 hours post admission' (209). Further information on the methods used for the identification of infection is described in chapter 2.4.1.

The secondary outcomes evaluated were in hospital mortality at ≤48 hours (early) and >48 hours (late) post injury, the presence of multiple organ failure, episodes of venous thromboembolism (VTE), stroke and myocardial infarction, ventilator free days (VFD), critical care length of stay (LOS) and total hospital LOS. The development of organ failure was assessed daily using the Sequential Organ Failure Assessment (SOFA) score. Single organ failure (SOF) was defined as a SOFA score of ≥3 in one organ system during a 24 hour period. Multiple organ failure (MOF) was defined as SOF in two or more organ

systems during a 24 hour period (176). Organ failure scoring systems are described in more detail in chapter 2.4.2.

VTE, stroke or myocardial infarction were diagnosed by the clinical team independent of the researcher. The presence of VTE was confirmed by either ultrasound scan (for deep vein thrombosis) and/or computed tomography pulmonary angiography (CTPA) (for pulmonary embolism). Stroke was confirmed using CT scan and the diagnosis of myocardial infarction was made utilising ECG, echocardiogram and troponin T and I tests. Patients were followed until hospital discharge, transfer or death.

6.3.4 Data analysis

Statistical analysis was performed using GraphPad PRISM v5 (GraphPad Software Inc, San Diego CA USA) and SPSS v21 (IBM Corporation, Armonk, NY, USA). Mann Whitney U test or Kruskal Wallis test were used to analyse non-parametric data and expressed as median (interquartile range). Parametric data was analysed with Students t-test or ANOVA, and expressed as mean (95% confidence intervals). Percentages were analysed using Chi square or Fisher's exact tests.

Multivariable linear and binary logistic regression models were created to identify if TXA was independently associated with infection and other outcomes. Initially, univariate statistical analysis was performed to examine the unadjusted effects of potential predictor variables. There were a large number of variables to be entered into the models. Following statistical advice those variables achieving a significance of $p<0.2$ were added to the multivariate regression models for stepwise regression analysis.

6.4 Results

In the two-year period, 456 patients were admitted to critical care following traumatic injury and were initially included in the study. Of these, 71 patients had an ISS <16 and were subsequently excluded, leaving 385 patients in the study. 160 patients (42%) received TXA as part of the major hemorrhage protocol within three hours following injury in either PHC or ED (Table 6.1).

Table 6.1 Admission demographics, injury characteristics and transfusion requirements: all patients

	All no TXA (n=225)	All TXA (n=160)
PHC scene to ED arrival (mins)^	56 (54-59)	54 (51-57)
Age^	40 (38-43)	42 (39-44)
Male (%)	82	78
Blunt (%)	93	84
ISS^	29 (27-30)	33 (31-35)*
AIS≥3 head (%)	64	65
GCS^	10 (10-11)	10 (9-10)
SBP^(mmHg)	127 (123-131)	102 (97-107)*
BD^	3 (2.7-4)	7.7 (5.8-7.5)**
INR^	1.1 (1.1-1.1)	1.3 (1.2-1.3)**
Time to OR/IR (mins)~	120 (47-306)	54 (30-120)**
Transfusion in first 24H from injury:		
PRBC (units)^	2 (1-3)	7 (6-8)**
FFP (units)^	1 (1-2)	5 (4-6)**
PLTS (units)^	0 (0-0)	1 (0-1)*
CRYO (units)^	0 (0-0)	1 (1-2)*
Crystalloid (mls)^	643 (474-812)	942 (752-1133)*

Values are expressed as ^mean (95% Confidence Intervals), ~median (IQR) or %. No shock = BD<6 mEq/L, Shock = BD≥6 mEq/L, PHC: Pre hospital care, ED: Emergency department, ISS: Injury severity score, AIS: Abbreviated injury score, GCS: Glasgow Coma Score, SBP: Systolic blood pressure, BD: Base deficit, PRBC: Packed red blood cells, FFP: Fresh frozen plasma, PLTS: Platelets, CRYO: Cryoprecipitate, OR: Operating Room, IR: Interventional Radiology. * indicates p <0.05 and ** p<0.01 when comparing two groups.

6.4.1 The effect of TXA in all patients

Overall, patients who had TXA were older, suffered more penetrating trauma and were found to have a significantly higher ISS than those in the no-TXA group (Table 6.1).

Patients who received TXA were more shocked (p<0.01) and more coagulopathic (p<0.01) on admission to hospital. Time to haemorrhage control for patients receiving TXA was half that of those in the no-TXA cohort (p<0.01). There was a three-fold increase in PRBC transfusion and a five-fold increase in FFP administration for patients in the TXA group (Table 6.1). For all patients infection rates were high, with no significant differences between the two groups (No TXA: Infection - 50% vs. TXA: Infection - 56% ns) (Table 6.2). Unadjusted mortality rates between the two groups were the same. The incidence of MOF was lower for those who received TXA compared to those who did not, however this did not achieve significance (No TXA: 37% vs. TXA: 30% ns).

Table 6.2 Clinical outcomes: all patients

	All no TXA (n=225)	**All TXA (n=160)**
Infection (%)	113 (50)	89 (56)
Mortality≤48H (%)	19 (8)	12 (8)
Mortality>48H (%)	18 (8)	17 (11)
Respiratory failure (%)	56 (26)	42 (27)
CVS failure (%)	103(47)	81(52)
CNS failure (%)	87(40)	43(27)
Coagulation failure (%)	5(2)	5(3)
Hepatic failure (%)	2(1)	5(3)
Renal failure (%)	9(4)	9(6)
MOF (%)	82(37)	46(30)
VTE (%)	9(4)	8(5)
Stroke (%)	3 (1)	5 (3)
M. Infarction (%)	3 (1)	3 (2)
28/7 VFD[†]	23 (18-27)	22 (14-26)*
CC LOS[†]	7 (3-12)	10 (4-18)*
Hospital LOS[†]	27 (14-40)	30 (16-49)*

Values are expressed as [†]median (IQR) or n (%). No shock = BD<6 mEq/L, Shock = BD≥6 mEq/L, MOF: Multi-organ failure, VTE: Venous thromboembolism, M. Infarction: Myocardial infarction, 28/7 VFD: 28 day ventilator free days, CC LOS: Critical care length of stay. * indicates p <0.05 when comparing two groups

6.4.2 The effect of TXA and shock

One hundred and twenty eight patients were in shock (BD ≥6 mEq/L) on arrival to the ED and of these, 84 (65%) patients were given TXA (Table 6.3). Those in the TXA group were more hypotensive on arrival to hospital (p=0.01) and more severely injured. Administration of PRBC and FFP transfusions was almost 50% higher for patients who received TXA compared to those who did not (Table 6.3).

Table 6.3 Admission demographics, injury characteristics and transfusion requirements: shocked and non-shocked patients

	Shock no TXA (n=47)	Shock TXA (n=84)	No shock no TXA (n=178)	No shock TXA (n=76)
PHC scene to ED arrivalˆ (mins)	55 (51-60)	55 (49-60)	56 (53-59)	53 (49-58)
Ageˆ	38 (33-43)	39 (36-43)	43 (18.9)	41 (18.6)
Male (%)	80	81	86	80
Blunt (%)	90	84	88	85
ISSˆ	31 (29-35)	35 (32-38)	27 (26-29)	31 (27-33)*
AIS≥3 head (%)	55	52	67	46*
GCSˆ	9 (8-10)	9 (8-11)	10 (9-11)	12 (11-13)*
SBPˆ(mmHg)	109 (100-118)	94 (87-102)*	132 (127-135)	110 (103-118)*
BDˆ (mEq/L)	10 (9-12)	12 (11-13)	1.4 (1.1-1.9)	2 (1.3-2.6)
INRˆ	1.2 (1.1-1.3)	1.3 (1.1-1.4)	1.1 (1.0-1.1)	1.1 (1.1-1.2)*
Time to OR/IR (mins)~	62 (32-454)	48 (30-113)	120 (55-300)	64 (33-133)*
***Transfusion in first 24H*:**				
PRBC (units)ˆ	6 (3-8)	10 (8-12)*	1 (0-1)	5 (4-6)*
FFP (units)ˆ	4 (2-6)	7 (5-8)*	1 (0-1)	4 (3-5)*
PLTS (units)ˆ	1 (0-1)	1 (1-2)*	0 (0-0)	1 (0-1)*
CRYO (units)ˆ	1 (0-1)	2 (1-2)*	0 (0-0)	1 (0-1)*
Crystalloid (mls)ˆ	730 (165-1315)	710 (457-967)	610 (418-705)	900 (636-1258)

Values are expressed as ˆmean (95% Confidence Intervals), ~median (IQR) or %. Shock = BD≥6 mEq/L, No shock = BD<6 mEq/L, PHC: Pre hospital care, ED: Emergency department, ISS: Injury severity score, AIS: Abbreviated injury score, GCS: Glasgow Coma Score, SBP: Systolic blood pressure, BD: Base deficit, PRBC: Packed red blood cells, FFP: Fresh frozen plasma, PLTS: Platelets, CRYO: Cryoprecipitate, OR: Operating Room, IR: Interventional Radiology. * indicates p <0.05 when comparing two groups

Incidence of infection was high but there were no significant differences between the groups (No TXA: 62% vs. TXA: 54%, ns) (Table 6.4). Early unadjusted mortality rates for those who had TXA were lower (Table 6.4). There was significantly less respiratory failure following TXA administration, and rates of MOF were significantly lower for those who had TXA (No TXA: 46% vs. TXA: 29%, p=0.02). There was however a four-fold increase in thromboembolic events in the TXA group (No TXA: 2% vs. TXA: 8%, p<0.01).

Table 6.4 Clinical outcomes: shocked and non-shocked patients

	Shock no TXA (n=47)	Shock TXA (n=84)	No shock no TXA (n=178)	No shock TXA (n=76)
Infection (%)	29(62)	45(54)	85(48)	43(57)
Mortality≤48H (%)	8 (15)	9 (11)	10 (6)	4 (5)
Mortality>48H (%)	4 (9)	9 (11)	15 (9)	7 (9)
Respiratory failure (%)	19(39)	20(24)*	37(22)	22(29)
CVS failure (%)	28(57)	51(65)	75(45)	30(40)
CNS failure (%)	15(31)	20(25)	72(43)	23(30)
Coagulation failure (%)	3(6)	4(5)	2(1)	1(1)
Hepatic failure (%)	2(4)	5(6)	0(0)	0(0)
Renal failure (%)	4(8)	7(9)	5(3)	2(3)
MOF (%)	22(46)	24(29)*	60(36)	22(29)
VTE (%)	2(2)	7(8)*	7(4)	1(1)
Stroke (%)	2 (4)	4 (5)	1 (1)	1 (1)
M. Infarction (%)	2 (4)	3 (4)	1(1)	0
28/7 VFD†	17 (5-23)	20 (16-26)*	24 (18-27)	25 (17-26)
CC LOS†	11 (4-18)	12 (7-20)	7 (3-10)	7 (3-13)
Hospital LOS†	30 (16-50)	30 (10-45)	18 (9-31)	26 (14-47)

Values are expressed as †median (IQR) or n (%). No shock = BD<6 mEq/L, Shock = BD≥6 mEq/L, MOF: Multi-organ failure, VTE: Venous thromboembolism, M Infarction: Myocardial infarction, 28/7 VFD: 28 day ventilator free days, CC LOS: Critical care length of stay. * indicates p <0.05 when comparing two groups

Of the 254 patients who were not shocked on arrival, 76 (30%) were given TXA (Table 6.3). Furthermore there was a 50% decrease in time to haemorrhage control for those who received TXA in this cohort (No TXA: 120 minutes vs. TXA: 64 minutes, $p<0.01$) and there was a five-fold increase in PRBC use and four times greater FFP transfusion administered to the TXA group (Table 6.3).

There were no significant differences in the development of infection between the two groups in the non-shocked cohort (No TXA: Infection - 48% vs. TXA: Infection - 57% ns) (Table 6.4). Unadjusted early and late mortality rates between the two groups were similar. There was a trend to a decreased incidence of MOF in the TXA group however this did not reach significance. Finally, there was no significant increase in the development of thromboembolic events for non-shocked patients irrespective of TXA administration.

Multivariate analysis was used to identify if TXA was independently associated with TXA and other clinical outcomes. Univariate analysis of all admission variables (from Tables 6.1 and 6.3) was performed to determine factors for multivariable analysis (Table 6.5). Logistic regression evaluated the effect of TXA on binary outcomes (infection, MOF, mortality, VTE, stroke and myocardial infarction). The effect of TXA on continuous outcomes (ventilator free days, critical care length of stay and hospital length of stay) was analysed using multivariable linear regression (Table 6.6).

Table 6.5 Clinical variables significantly associated with outcome in univariate analysis

Dependent variable	Independent variable	All patients	Shock cohort	No shock cohort
Infection	ISS	**0.08**	Ns	Ns
	BD	**<0.01**	**0.02**	**0.01**
	PRBC	**0.04**	Ns	**0.04**
	TXA	**0.07**	**0.14**	**0.90***
	GCS	Ns	**0.08**	**0.01**
	INR	Ns	**0.18**	Ns
Mortality	Age	**<0.001**	Ns	**<0.01**
	GCS	**<0.001**	**0.05**	**0.10**
	Blunt mechanism	**0.01**	Ns	Ns
	TXA	**0.08**	**0.02**	**0.19**
	PRBC	**<0.01**	**0.03**	**<0.01**
	BD	**<0.001**	**0.16**	Ns
	INR	Ns	Ns	**0.07**
	ISS	Ns	Ns	**0.17**
	TTOR/IR	Ns	**0.09**	Ns
MOF	ISS	**<0.01**	**<0.01**	**0.04**
	BD	**<0.001**	Ns	Ns
	TXA	**0.06**	**<0.01**	**0.19**
	PRBC	**0.01**	**0.04**	Ns
	GCS	Ns	Ns	**<0.01**
	TTOR/IR	Ns	**0.01**	Ns
VTE	TXA	**0.42***	**0.16**	**0.32***
	Gender	**0.20**	**0.05**	Ns
	Blunt mechanism	Ns	**0.08**	Ns
Stroke/MI	TXA	**0.24***	**0.21***	**0.83***
	ISS	Ns	Ns	**0.16**
	BD	Ns	**0.19**	Ns
	Gender	**0.20**	Ns	Ns

GCS: Glasgow coma score, TXA: Tranexamic Acid, PRBC: Packed red blood cells, BD: Base Deficit, INR: International normalised ratio, ISS: Injury severity score, TTOR/IR: Time to operating room or interventional radiology, MOF: Multi-organ failure, VTE: Venous thromboembolism, MI: Myocardial infarction. *Following statistical advice, multivariate analysis of TXA on all outcomes was included in the table whether p<.20 or not, ns: not significant.

Multivariable logistic regression revealed that TXA was not independently associated with a reduction in infection for any of the cohorts within this study (Figure 6.1).

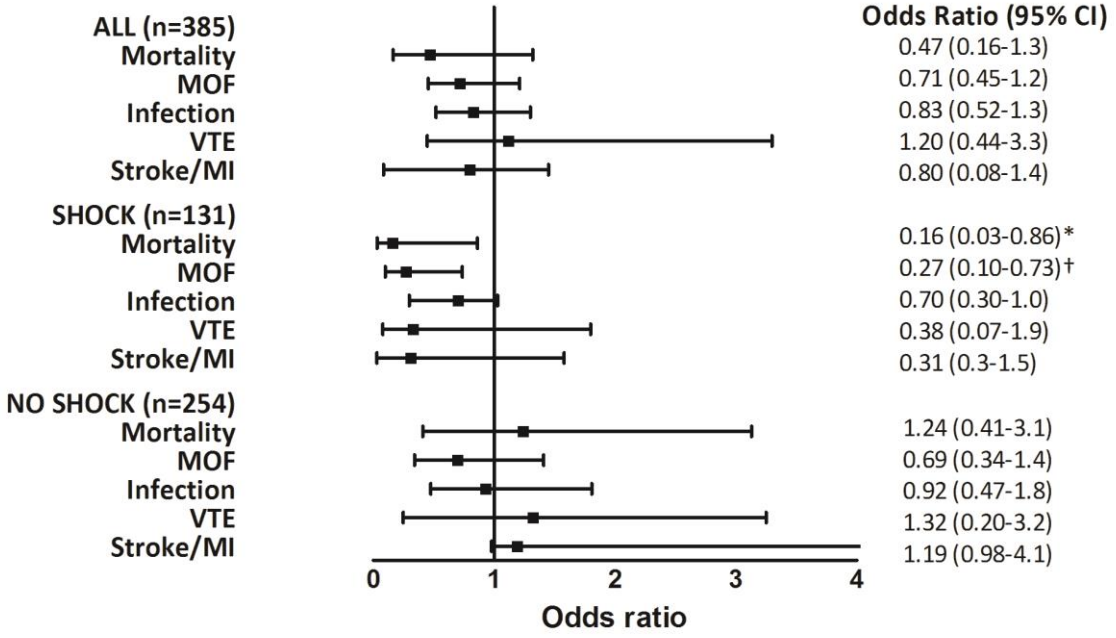


Figure 6.1. The effect of TXA on binary outcomes in all, shock and no-shock cohorts. Forest plot shows odds ratio and 95% confidence intervals for the effect of TXA on each binary outcome. MOF: Multiple organ failure, VTE: Venous thromboembolism, MI: Myocardial infarction. †p=0.03, * p=0.01.

There was a trend to a protective relationship between TXA and infection in the shocked cohort. Although this didn't achieve significance in regression analysis, the odds ratio of 0.7 suggested a beneficial association which may have become significant with a larger cohort. TXA was independently associated with a reduction in MOF and mortality in shocked patients (Figure 6.1). Furthermore, shocked patients also had a greater number of ventilator free days after TXA use (Table 6.6). However, TXA was not independently associated with any change in infection, mortality, MOF or other outcomes in the non-shocked cohort, (Figure 6.1).

Table 6.6 Multivariate linear regression: the effect of TXA on continuous outcomes

	β-coefficient	95% CI	p value	R square
All cohort				
VFD	-.079	-2.5 - .08	0.06	0.38
CCLOS	.081	-.35 – 4.7	0.09	0.24
HLOS	0.19	-4.4 – 6.7	0.69	0.19
Shocked cohort				
VFD	3.80	4.1 – 7.2	0.02	0.51
CCLOS	4.67	-1.1 - 10.4	0.11	0.21
HLOS	-3.88	-17.2 – 9.4	0.56	0.17
No shocked cohort				
VFD	-.844	-2.8 – 1.2	0.41	0.30
CCLOS	1.35	-2.0 – 4.7	0.43	0.14
HLOS	.111	-.47 – 14.2	0.07	0.14

Shock = BD≥6 mEq/L, no shock = BD<6 mEq/L, VFD: ventilator free days, CCLOS: critical care length of stay, HLOS: hospital length of stay.

6.5 Discussion

This prospective study has characterised the relationship between TXA use in severely injured civilian patients and infection. TXA administration did not have an independent relationship with the development of infection in any of the study cohorts. TXA use was associated with improved mortality and organ failure in patients presenting with shock. Yet there were no clear outcome benefits identified for patients who arrived to hospital without shock. However there was no independent association between TXA use and adverse events in this study.

Overall infection rates were high for all cohorts within the study. TXA is known to inhibit the activity of the plasminogen-plasmin pathway (206) resulting in a reduction of both inflammation and fibrinolysis (186, 191, 244). Whilst an independent relationship was

not observed in this relatively small sample of participants, there was a trend to reduced infection following TXA only in shocked patients, where the mechanism of action may have greatest utility.

TXA administration was associated with reduced rates of organ failure, which is known to be associated with poor outcomes (23). The significantly lower rates of respiratory failure in shocked patients who received TXA were consistent with the increased number of ventilator free days. Although the incidence of MOF in the overall cohort was high, patients were more severely injured than in the previous TXA studies (64, 203). Following TXA administration there was a reduction in MOF in shocked patients. This was despite the presence of admission coagulopathy and increased rates of blood transfusion, both of which are reportedly associated with the development of MOF (68, 69, 175). Trauma related plasmin generation in bleeding patients is known to produce proinflammatory responses which may be responsible for the development of MOF (240). The anti-inflammatory effects of TXA may be responsible for the observed reduction in single and multiple organ failure associated with shock and hemorrhage.

TXA use was also associated with decreased early mortality and was protective for adjusted all-cause mortality in shocked patients. The non-significant increase in late crude mortality may be the result of improved early survival in a more severely injured, shocked cohort of patients who presented with lower systolic blood pressures and had greater transfusion requirements. Overall the beneficial effect of TXA on mortality in shocked trauma patients reported previously was also evident early in this civilian trauma population (64). After adjusting for confounding variables in the non-shocked cohort, there was no effect of TXA on infection and mortality, and only a non-significant trend towards reduction in MOF.

Although VTE was more common in patients who received TXA, this again may be due to its administration to a more severely shocked population with longer initial survival

rates. There may also have been a delay in instituting thomboprophylaxis in a more severely injured patient group. However there was no statistically significant relationship in the multivariate analysis which if anything showed a trend to a reduced risk of VTE after TXA in shocked patients. This is consistent with the reduced thrombotic event rates observed in the CRASH 2 study (64).

In this study BD was used as a marker of tissue hypoperfusion, rather than systolic blood pressure which is utilised in the major haemorrhage protocol. Systemic tissue hypoperfusion is known to drive fibrinolysis in trauma (183). Whilst BD is available as a point of care test in many EDs, it is unlikely to be used in PHC and waiting for BD results could potentially delay administration of TXA. However, clinical markers such as blood pressure are known to be poor indicators of the degree of systemic hypoperfusion (207, 208). In the CRASH 2 subgroup analysis, the most pronounced benefits of TXA were seen in patients with SBP<75mmHg (64). This threshold would potentially miss a large number of patients with significant hypoperfusion. Further work is needed to identify parameters for use in clinical practice associated with clinically important fibrinolysis in trauma.

There are a number of limitations to this study. Primarily it was conducted in a single setting, albeit a large urban major trauma centre. The numbers within the sub cohorts were relatively small and the non-significant infection results may have become significant with a larger number of participants, especially for those in the shocked group. This represents an avenue for further study, especially when considering the relationships found in previous chapters between shock, coagulation changes and infection. Finally, data was not collected on the use of or adherence to thomboprophylaxis guidelines during the study, which may have assisted with the analysis of VTE. Despite the limitations, the findings give a clear signal for using TXA in severely injured, shocked civilian patients in order to benefit clinical outcomes.

6.6 Conclusion

In conclusion, this is the first study to show that the use of TXA as part of a major hemorrhage protocol provides outcome benefits specifically for severely injured shocked patients. In particular, TXA was independently associated with a reduction in MOF and mortality in patients presenting to hospital in a hypoperfused state. An independent relationship between TXA and infection was not observed, however these results will serve as a basis for future work. This includes prospective studies to better understand the relationship and mechanistic pathways between inflammation, infection and TXA use in a larger cohort of shocked patients.

CHAPTER SEVEN CONCLUSIONS, STRENGTHS AND LIMITATIONS OF THE RESEARCH

7.1 Summary of thesis and suggestions for future work

The research I have described in this thesis has achieved the aims proposed in chapter one. The findings have provided novel contributions to the existing evidence on predictors of infection after trauma. Chapter three described the burden of infection for severely injured civilian patients and demonstrated its association with admission hypoperfusion which has not been previously reported. In chapter four the relationship between changes in immune cell responses and infection was confirmed. Lymphopenia prolonged to day four post injury was strongly predictive of the development of infectious complications. Persistent lymphopenia still present at four days post injury has previously been linked with increased mortality (162). I was able to add to this evidence by confirming that lymphopenia at day five is also associated with mortality after severe injury. Chapter five demonstrated that coagulopathy, specifically activation of anticoagulant and fibrinolytic pathways, which was not corrected by 24 hours post injury was associated with infection. Furthermore the degree of coagulation dysfunction directly correlated with the incidence of infection. Lastly, chapter six was the first study to evaluate early antifibrinolytic therapy and its effect on infection and other clinical outcomes in a severely injured cohort. There was no significant beneficial relationship between TXA and the development of infection. However it was associated with improved MOF and mortality for patients presenting to hospital in a hypoperfused state, and thus adds to the current understanding of the utility of TXA in trauma.

This research has allowed me to demonstrate that infection is a significant cause of morbidity following severe injury, and the burden for this population was greater than previously appreciated. Hospital associated infections are of concern for all healthcare providers and many infection prevention and control guidelines are advocated (121,

213, 245). However in this research, drivers or predictors of infection were found to be related to the patients presentation in the initial period after severe injury. The predominant early characteristic predictive of infection was admission hypoperfusion. Once admitted to hospital, patients had significant alterations to their coagulation and immune systems in the first few hours and days from injury which were subsequently associated with the development of infection.

My findings suggest that there is the potential for an early clinical window where modulation of the coagulation and immune system changes could provide an opportunity to reduce the burden of infection. Antifibrinolytic therapy is already utilised in clinical trauma haemorrhage management (193) and may play a beneficial role in reducing infection in a larger patient population. Further investigation into optimal resuscitation and transfusion practice which may help to modify coagulation-inflammation pathways is warranted.

In the studies described within this thesis, patients who developed infection in critical care had longer lengths of stay. This prolonged their post injury recovery and may have had negative effects on the patient experience in hospital. Infection may also have a detrimental impact on post-discharge recovery or readmission rates. Infectious complications after severe injury also worsens economic burden. The increased costs associated with treating infections and prolonged length of stay following trauma are reported to be double that of non-infected patients (73, 136). Future work should focus on timely haemorrhage control and correction of coagulopathy which may result in reduced infections after trauma, improve patient recovery and decrease economic burden.

7.2 Strengths and limitations of the research

This thesis reports the findings from four prospective studies, conducted at a large urban major trauma centre. To best evaluate those most at risk of infection, severely injured critical care patients were chosen as the study population. Data was collected contemporaneously on a daily basis. This resulted in complete, quality data capture which measured a number of complex interventions and outcomes. The studies used data definitions that were consistent with internally agreed standards (such as those for infection or organ failure), helping to minimise variance and increase the generalizability of findings.

Robust multivariate models were used to establish strong relationships between a number of significant trauma variables and infection. The presence of admission shock, fibrinolysis at 24 hours post injury and lymphopenia to day four after admission were all strongly associated with the development of infection. Therefore, the findings have provided potential clinical indicators for trauma clinicians to identify those patients at risk of infection following severe injury. Finally, there was a high incidence of infection in severely injured patients in all of the studies. This significant finding suggests that infection has the potential to be used as sensitive measure of outcome in other trauma research studies and clinical trials.

I have acknowledged a number of limitations within this thesis. Whilst multivariate analysis reduced the effect of confounding variables, many of the predictions of infection were based on associations in relatively small study populations. Findings implicating haemorrhage, coagulation and antifibrinolytics in the development of infection would benefit from substantiation in future studies with larger numbers and greater statistical power. Other than the use of TXA and blood products, the role of acute phase care was not investigated therefore further research examining early intervention for trauma patients known to be or suspected of bleeding and the effect on infection is warranted.

There was a strong association between unresolved lymphopenia and infection, however the mechanistic link between the two was not investigated and requires further immunological characterisation. Furthermore, sampling immune cell counts for a longer time period may have yielded further results in patients who developed infection later in their clinical course associated with prolonged or persistent organ failure (PICS).

Bacterial pathogens are increasingly resistant to broad spectrum antibiotics and the development of new antibiotics is limited (131, 246, 247). Adherence to antibiotic stewardship is a key focus for military trauma research (134, 248). In my studies, the majority of trauma patients were given prophylactic antibiotics. No differences were found in the development of infection between those who received prophylaxis from admission and those who did not. However further investigation of the utility and adherence to prophylactic antibiotic guidance may add to the characterisation of trauma infections, and further work with civilian patients is warranted.

Finally, I did not compare outcomes following discharge from hospital between patients who did and did not develop infection. The effects of infection developed in critical care following injury may be far reaching. Understanding longer term physical and psychological consequences of infection may provide important patient and system performance outcome measures.

7.3 Conclusion

This thesis has completed all of the study aims and has characterised important predictors of infection following trauma. Infection continues to be a significant burden for severely injured patients, resulting in adverse outcomes whilst in hospital. Admission shock, altered coagulation at 24 hours and prolonged lymphopenia following injury were found to be independent predictors of infection. Early use of antifibrinolytics had no significant impact on infection rates, however was associated with reduced mortality and organ failure. Further research into the effects of early coagulation and immune cell modulation on the development of infection is needed.

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